ROOM TEMPERATURE PHOSPHORESCENCE: SOME DIAGNOSTIC STUDIES IN ITS APPLICATION TO BIOCHEMICAL AND DRUG ANALYSIS

BY

RICKY P. BATEH

A DISSERTATION PRESENTED TO THE GRADUATE COUNCIL
OF THE UNIVERSITY OF FLORIDA IN
PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA
1982

Scientific research is a social activity. . . . To understand the nature of science, we must look at the way scientists behave towards one another, how they are organized, and how information passes between them. The young scientist does not study formal logic, but learns by imitation and experience a number of conventions that embody strong social relationships--learns to play a role in a system by which knowledge is acquired, sifted, and eventually made public property.

John Ziman

When we abandon the pursuit of truth for any reason, whether it is because it is dangerous or because we are lazy or for any other reason, then we become parasites, robbing society and giving nothing in return. This is why academic people have always to be so hard on each other. You will find out that we are ruthless in exposing each others' errors and eliminating those of our number who let the standard fall. We have to because the scholar who abandons the truth is a menace to our whole community.

Arthur Lewis

This dissertation is dedicated to my wonderful family. My father, forever inquisitive, has relayed to me the importance of communication between professional personnel and the layperson. My mother, always supportive of my career decisions, has bestowed upon me the ultimate power of contemplation. My brother, a friend indeed, has shown continuous dedication to my professional achievements. Last but not least, my sister, brother-in-law and niece have provided me with the happiness necessary to attain the highest spiritual level.

ACKNOWLEDGEMENTS

The author would like to thank Dr. J. D. Winefordner for his support and encouragement throughout these research endeavors. His liberal attitude towards graduate research has been favorably applied towards my development as a practical scientist. The JDW group has been, and always will be, an interesting group with which to work. The author would also like to acknowledge the assistance of the three postdoctoral factoti, Jimmie Ward, Edward Voigtman and Ben Smith. He is most grateful to Jeanne Karably for preparation of this work in its present form and to Jeff Pate for preparation of the artwork.

TABLE OF CONTENTS

		PAGE
ACKNOWLEDGEMENTS		iv
ABSTRACT		vii
CHAPTER		
1	INTRODUCTION	1
2	OVERVIEW OF PHOSPHORESCENCE	3
	Introduction	3
	Studies in Low Temperature Phosphorescence	5
	Studies in Room Temperature Phosphorescence	7
	Diagnostics	10
	Instrumentation	10
	Procedural Considerations	10
	Summary	22
3	AN EVALUATION OF CELLULOSE AS A SUBSTRATE MATERIAL FOR ROOM TEMPERATURE PHOSPHORESCENCE	23
	Introduction	23
	Experimental	24
	Reagents and Materials	24
	Procedure	24
	Results and Discussion	26
	Background on Papermaking	26
	Diagnostical Studies on Cellulose Products	28
	Phosphorescence background	28
	Fibrillation study	33
	Filter paper comparison	33
	Filter-paper lots comparison	36
	Conclusion	36
	Note	36
4	BIOCHEMICAL AND DRUG ANALYSIS BY ROOM TEMPERATURE PHOSPHORESCENCE	39
	Introduction	39

Biochemical/Drugs 40
Pharmaceutical Formulations 59
Experimental 61
Reagents and Materials 61
Sample Preparation 61
Biochemicals/drugs 61
Pharmaceutical formulations 66
Procedure 66
Results and Discussion 72
Biochemical/Drug Survey 72
Xanthines 72
Indoles 75
Benzoic acid derivatives 78
Others 84
Pharmaceutical Analysis 110
Xanthines 110
Analgesics 110
Others 114
Summary 114

5 SUMMARY AND FUTURE CONSIDERATIONS 119

APPENDICES

1 GLOSSARY FOR CHAPTER 3 121

2 ANCILLARY STUDIES 123

Herbicide Survey 123
Introduction 123
Procedure 123
Results and Discussion 123
Therapeutic Drug Monitoring 125
Introduction 125
Reagents and Materials 125
Procedure 126
Results and Discussion 126

3 COMPANY LIST 128

4 DATA FOR LINEAR REGRESSION ANALYSIS 130

REFERENCES 131

BIOGRAPHICAL SKETCH 135

Abstract of Dissertation Presented to the Graduate Council of the University of Florida in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy

ROOM TEMPERATURE PHOSPHORESCENCE: SOME DIAGNOSTIC STUDIES IN ITS APPLICATION TO BIOCHEMICAL AND DRUG ANALYSIS

By

Ricky P. Bateh

August, 1982

Chairman: J.D. Winefordner
Major Department: Chemistry

Room temperature phosphorescence (RTP) involves the measurement of phosphorescence irradiated by molecules illuminated with a source of ultraviolet light. In RTP, analyte molecules are adsorbed onto solid substrate materials, such as filter paper and silica gel, and analyzed for their characteristic phosphorescence emission.

In these studies, RTP test methodologies and applications of RTP are evaluated for their effectiveness in demonstrating the analytical utility of the technique. Improvements are made in sample-compartment design and in sampling procedures currently used in RTP. A list of compounds exhibiting RTP is made to add to the present compilation of molecules known to phosphoresce at room

temperature. Trends in phosphorescence intensities are indicated for groups of "homologous" compounds; generalizations are made for such trends, but no mechanisms are described. Finally, RTP is used for the determination of a variety of drugs in pharmaceutical formulations, over-the-counter preparations and blood serum. While RTP has been shown to be a viable method of chemical analysis, real-sample applications have been limited. Thus, these latter studies represent significant accomplishments in RTP test methodologies. The procedures reported here are quite simple and specific for the determination of a variety of compounds in real samples.

CHAPTER 1
INTRODUCTION

In the past decade, room temperature phosphorescence (RTP) has received considerable attention as a method of analysis for biochemicals, drugs and polynuclear aromatic hydrocarbons. With the introduction of RTP as a new analytical technique, it is necessary to evaluate the procedure for appropriate test methodologies and applications. Several workers (1-3) have reviewed the fundamental interactions responsible for room temperature phosphorescence, some analytical methodologies and a variety of applications.

In a continuation of the recent work on RTP, this research was designed to optimize existing RTP methodologies, to evaluate the selection of an appropriate solid substrate for use in RTP, to compile a list of molecular species that exhibit room temperature phosphorescence under specified conditions and to evaluate the analytical utility of RTP in real-sample analysis. The first project involved a design-modification of the multiple-sampling bar device (4) and a rigorous evaluation of the sampling procedure used in RTP. The latter study resulted in a major change in procedure which allowed for a ∿3-fold reduction in analysis time (from ∿30 min to ∿10 min for 4 samples). Following the procedure-optimization study, an evaluation of

cellulose as a substrate material for RTP was made to facilitate selection of cellulose-based substrate materials which would enhance phosphorescence of analyte molecules. Once the RTP system was optimized, an in-depth study was initiated to establish a data base of molecules exhibiting RTP. In addition, several groups of "homologous" compounds were studied to determine the effects of a variety of substituents on phosphorescence intensity. In applying RTP to real-sample analysis, it was shown that RTP could be used quite-successfully in analyzing for a variety of active ingredients (drugs) in pharmaceutical formulations, over-the-counter preparations and in blood serum.

CHAPTER 2
OVERVIEW OF PHOSPHORESCENCE

Introduction

Luminescence is characterized by the emission of light following its selective absorption. Fluorescence and phosphorescence are two types of photoluminescence which can occur following excitation by any form of electromagnetic radiation.

In order to differentiate between absorption, fluorescence and phosphorescence processes, one should refer to the modern theory of molecular structure. Consider a molecule possessing a series of closely spaced energy levels that can be excited from a lower to a higher energy level by absorption of a discrete quantum of energy. A simplified Jablonski diagram (Figure 1) shows the processes which a molecule can undergo. Absorption can take place and results in a transition from the ground electronic state to a higher electronic state. These ground to excited singlet transitions are responsible for the ultraviolet-visible absorption spectrum of a molecule. During excitation, excess energy can be rapidly dissipated via radiationless decay (internal conversion) and the lowest vibrational level of the first excited state (S_1) is attained. The transition from the first excited singlet (S_1) to the

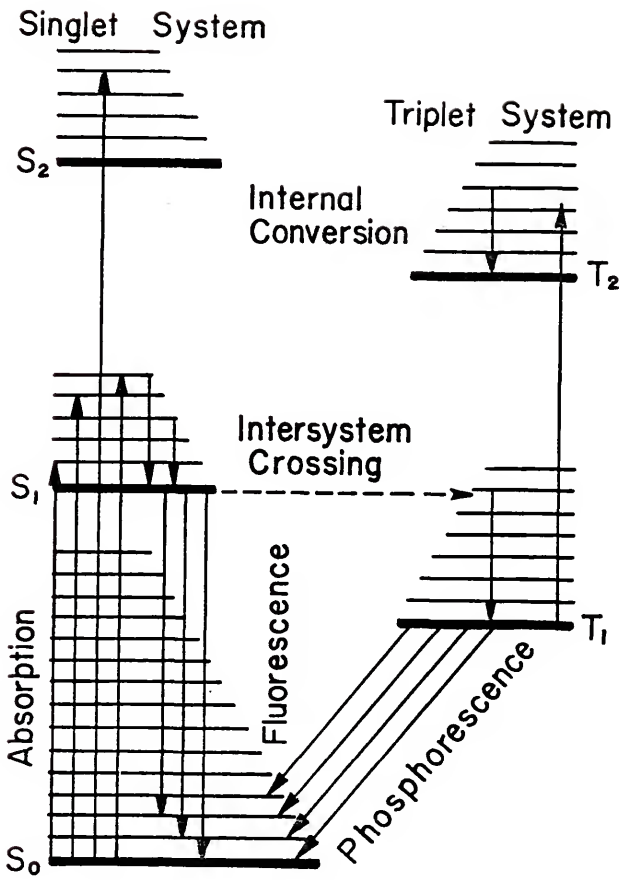


Figure 1. Simplified energy level diagram of a polyatomic molecule.

ground state (S_0) gives fluorescence. If a molecule in its excited state undergoes instead of internal conversion, intersystem crossing to the triplet state (T), then relaxation from the first excited triplet state (T_1) to the ground state (S_0) gives phosphorescence. In general, fluorescence takes place in a shorter time frame and at shorter wavelengths than phosphorescence. Molecules in the metastable triplet state are more susceptible to radiationless decay (collisional deactivation), and as a result, only molecules held in rigid media phosphoresce. For phosphorescence, two general categories of rigid media involve either dissolution of the analyte molecule in an appropriate solvent and freezing in liquid nitrogen (77 K) or adsorbing onto solid substrate materials such as silica gel and filter paper at room temperature (298 K).

Studies in Low Temperature Phosphorescence

The conventional approach to phosphorimetry involved the study of low temperature phosphorescence (LTP). In 1888, Wiedemann (5) first reported the phenomenon of phosphorescence by observing dyestuffs adsorbed in gelatin at 193 K. Some 55 years later, Lewis and Kasha (6) suggested that phosphorescence could be used as a means of chemical analysis. Kiers, Britt and Wentworth in 1957 (7) demonstrated the first use of phosphorescence as a method of analysis of organic molecules by showing that mixtures of phosphorescent molecules could be spectrally-resolved via selectivity brought about by differences in excitation and

emission wavelengths. By taking advantage of differences in luminescence lifetimes of the molecules, they were able to use temporal resolution as a further means of characterizing the molecules.

In the subsequent 10 years, several laboratories evaluated the use of LTP as an analytical technique by using a variety of Dewar flask systems (77 K) in immersion-cooling and conduction-cooling modes (8-14). Immersion cooling was the first choice because liquid nitrogen was transparent and non-luminescent in the wavelength range of 200-800 nm. However, there were numerous disadvantages to this configuration, the two most important of which being poor precision and accuracy of the measurement due to irreproducible positioning of the sample cell and the translucent nature of the frozen solution in the sample cell. Other disadvantages included light loss due to the multi-layered quartz system, flicker of excitation/emission radiation due to refractive index changes caused by convection of the coolant as it warmed, and additional flicker noise due to bubbling of the coolant at nucleation sites caused by dust or scratches inside the Dewar flask. Overall, the immersion cooling technique was a time-consuming, laborious method of analysis and improvements were sought in the conduction cooling method.

Early designs of conduction cooling systems performed poorly due to poor thermal contact between the sample cell and the copper rod and due to fogging of the viewing area.

In addition, a vacuum, necessary to attain the required low temperature, had to be broken to introduce a new sample. To compensate for these limitations, two new designs were recently evaluated (15,16). Although these designs facilitated sample introduction and decreased sampling time by ∿4-fold, the lowest temperature that could be attained was ∿85 K for the immersed copper mass and ∿100 K for the flow-through conduction cooling system. On the whole, low temperature measurements were difficult to make and the possibility of routine applications or automation of phosphorimetry using LTP was remote.

Despite the limited success of LTP, it could be to one's advantage to consider the more simple RTP system. The immediate advantage of a RTP system is the absence of cryogenic equipment necessary in LTP. While the simplicity of the RTP system could lend itself to automation, it is less sensitive but more selective than LTP.

Studies in Room Temperature Phosphorescence

The first observation of RTP of organic molecules adsorbed onto solid substrate materials occurred in 1941 where samples were placed in solid boric acid (17). The same phenomenon was also observed when samples were prepared in a rigid polymer matrix such as poly(methyl methacrylate) (18). In 1957, Szent-Györgyi (19) proposed spectrophosphorimetric analysis on paper and thin layer chromatograms by dipping the sheets in liquid nitrogen and then irradiating them with an ultraviolet light source. Roth, in

1967 (20), using a mercury lamp (254 nm) to irradiate chromatograms, observed luminescence lasting 3-10 sec after termination of the exciting light. While these observations suggested phosphorescence rather than long-lived fluorescence, no quantitative work was done. Schulman and Walling, in 1972 (21), rediscovered RTP when they observed phosphorescence radiating from a variety of ionic organic molecules adsorbed on paper, silica, alumina and other supports. This phenomenon was most pronounced when filter paper was the substrate material and the solvent was strongly acidic or basic. The heavy atom effect (addition of a "heavy atom," e.g., iodide or silver, to enhance phosphorescence intensities) was first studied by Kasha in 1952 (22) and applied to analytical phosphorimetry in 1963 by McGlynn and coworkers (23).

Early work in RTP involved the use of circular filter paper discs placed in sample holder tips (Figure 2) (24-26). The utility of RTP as an analytical tool had in some ways been hindered by the continued use of this method. The preparation of single samples on the tips was relatively time consuming in that each sample or blank had to be dried individually. Batch preparation of the samples was not always convenient since for reasons of precision, the timing of the drying and measurement of these samples was critical. In the past, additional lack of precision had been attributed to possible problems of positioning the filter paper discs and/or tips in the sample compartment of the instrument.

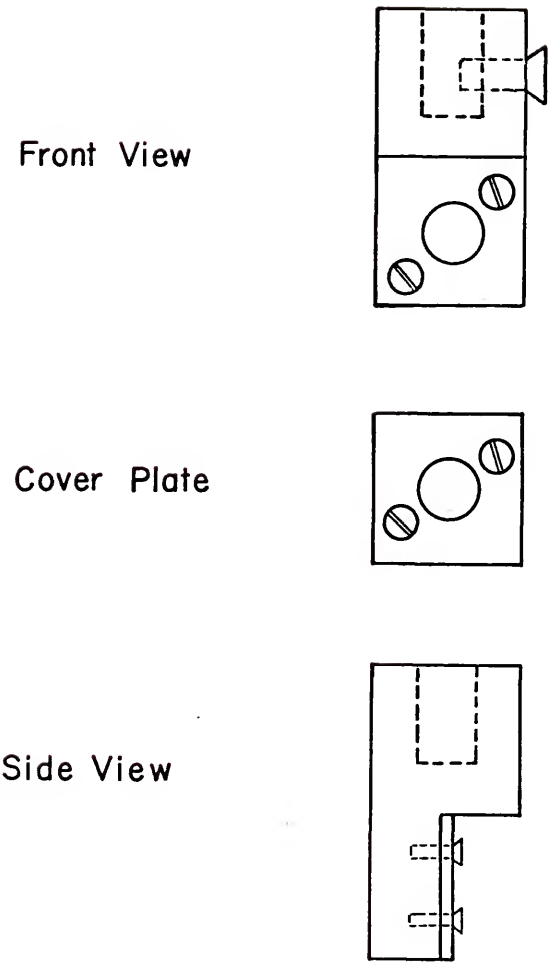


Figure 2. Sampling tip for room temperature phosphorescence studies.

In order to overcome the drying time and positioning problems and improve the uniformity of the treatment of multiple samples, a multiple-sampling bar (Figure 3) was evaluated (27). While this new device complements the sampling procedure in RTP quite well, there are additional methods to consider in evaluating its overall effectiveness in RTP. The present work has continued the evaluation of the multiple-sampling bar for a variety of applications in RTP.

Diagnostics

Instrumentation

All RTP measurements were made with a spectrofluorometer fitted with a xenon arc lamp, a laboratory-constructed phosphoroscope (Figure 4) (4) for bar-RTP (27) and a potted photomultiplier tube. A ratio photometer supplied high voltage to the photomultiplier tube in addition to serving as a dc amplifier. All line voltages were regulated with an ac regulator. A block diagram of the system configuration is shown in Figure 5. The components of the RTP system are listed in Table 2-1. All operating conditions were the same throughout all of the RTP studies.

Procedural Considerations

In order to compensate for some of the inefficiencies encountered with the original bar-RTP work, the sampling procedure and the position of the flush port were changed. The original sampling procedure consisted of placing filter paper discs ($\sim$0.25 in dia.), obtained by punching filter paper with a standard office paper punch, under the cover

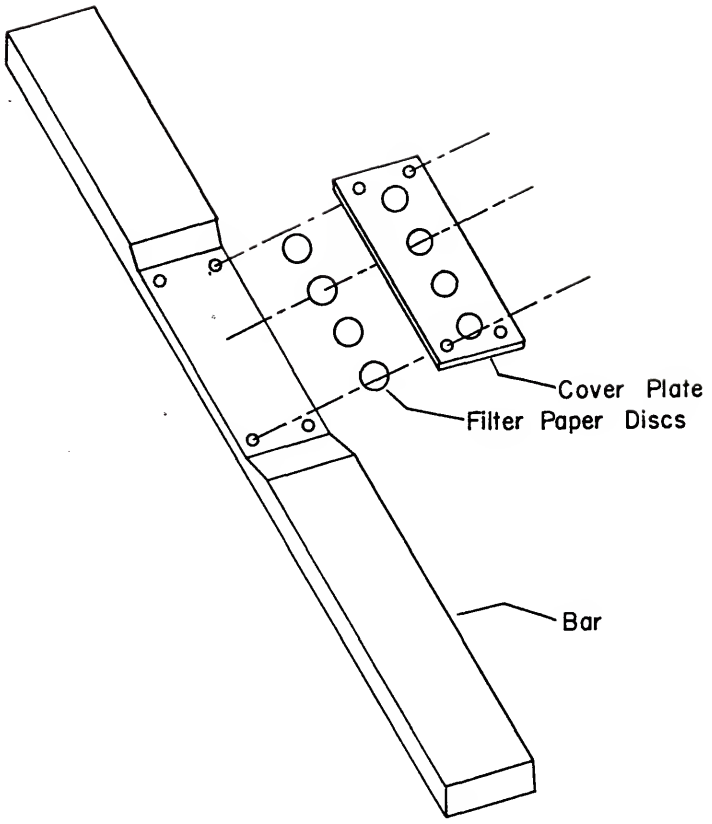


Figure 3. Multiple-sampling bar for room temperature phosphorescence studies.

Figure 4. Laboratory-constructed phosphoroscope assembly:

A. Base plate
B. Synchronous motor
C. Aligning block
D. Drive pulley
E. O-ring
F. Shutter can
G. Pulley
H. Ball bearing
I. Sample compartment housing
J. Slit holder
K. Slit

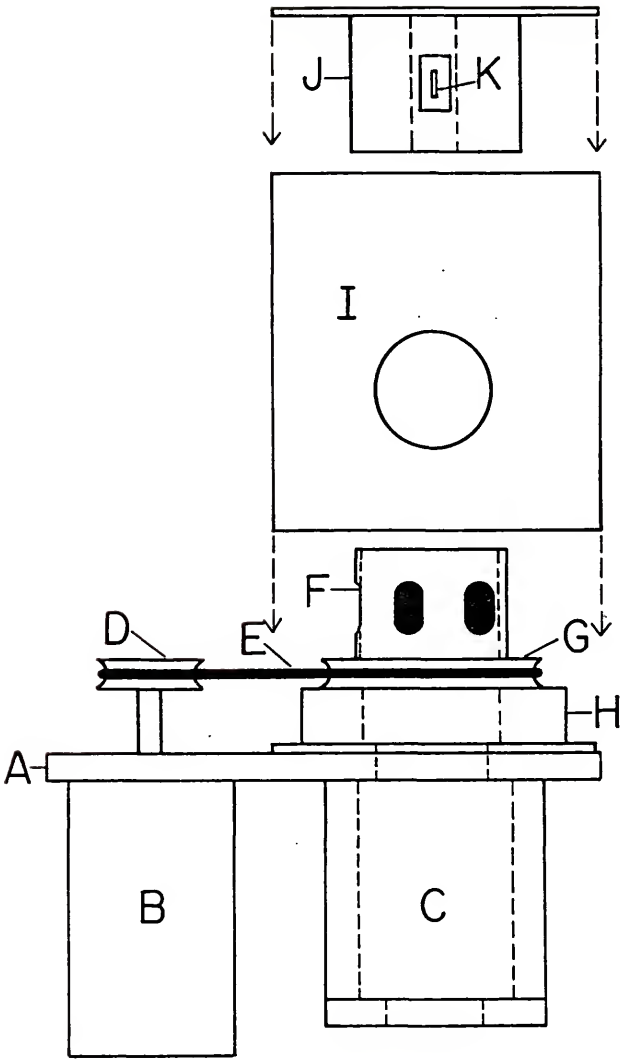

J
K
I
F
D
E
G
H
A
B
C

Figure 5. Block diagram of the room temperature phosphorescence instrumental system:

A. Lamp power supply
B. Xenon arc lamp
C. Quartz lens
D. Slit
E. Excitation monochromator
F. Sample compartment with phosphoroscope assembly
G. Emission monochromator
H. Potted photomultiplier tube
I. Ratio photometer
J. X-Y recorder

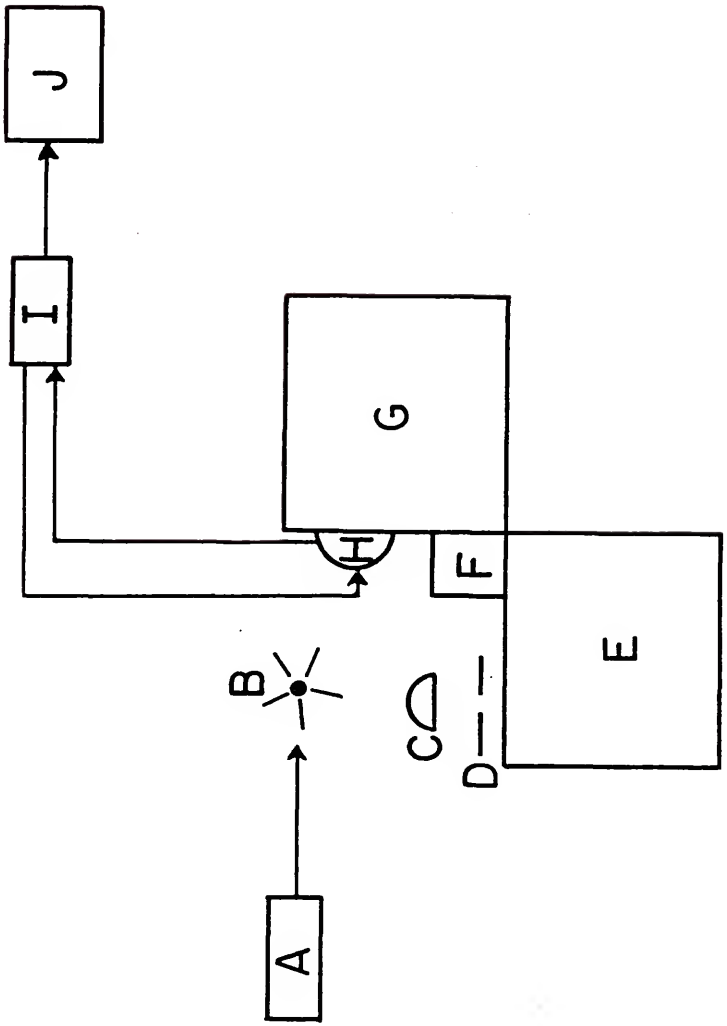

J
I
G
H
F
E
B
C
D
A

Table 2-1. Instrumentation for RTP Studies

Item	Model (Operating conditions)	Source
Lamp power supply	LPS 251 HR (7.5A, 20 V)	Schoeffel
Xenon arc lamp	901C-0011 (150 W)	Canrad-Hanovia
Spectrofluorometer	Aminco-Bowman SPF 4-8202 Slits- 4,3,2,2,3,2, mm	American Instrument Co.
Sample compartment	-----	American Instrument Co.
Phosphoroscope	Laboratory-constructed (200 Hz)	Reference 4
Sampling bar	Laboratory-constructed	Reference 27
Photomultiplier tube	1P21 (750 V)	Hamamatsu
Ratio photometer	4-8912 (R2, SV 100)	American Instrument Co.
X-Y recorder	1620-827	American Instrument Co.
ac regulator	ACR 3000	Sorenson

plate (with 4 holes each ∿0.25 in dia.) of the bar and then the cover plate was tightened into place on the bar with 4 screws. Samples were spotted onto the paper discs in 5 μL volumes with a Micro/pettor (Scientific Manufacturing Industries). After spotting, the bar was placed under an infrared lamp (12 min at ∿60°C with the lamp at a height of ∿8 in) so that the samples could dry. After drying, the bar was transferred to the sample compartment (equipped with a phosphoroscope can with a chopping rate of 200 Hz) and was allowed to equilibrate under a flow of dry argon gas (15 min at ∿20 L/min via a back-side flush). The drying and flushing steps were necessary to eliminate moisture and oxygen, both of which quench phosphorescence. Sample phosphorescence signal levels increased over a period of ∿14 min at which time a plateau was reached for ∿2 min. Measurements were made on this plateau.

In evaluating the previous procedure, it was thought that the use of the infrared lamp to dry the samples would alter the paper characteristics (see Chapter 3) and/or would affect the chemical stability of the analyte molecules. These effects have in fact been confirmed in one laboratory (28). It was found that in the presence of acid, base or heavy atom perturbers (Pb^{+2}, Tl^{+}, Ag^{+} and I^{-}), heating could cause oxidation of the hydroxyl groups on the surface of the cellulose substrate material (resulting in a greatly diminished RTP signal) or could cause oxidative breakdown of certain analyte molecules. Thus, in order to eliminate

such undesirable effects during lamp-drying, an alternative approach to dry the samples was investigated. First, the flush-gas inlet port was changed from the back side of the sample compartment to a front-surface flush position (Figure 6). Next, this modified configuration was compared to the lamp-dry/back-side flush configuration. It was found that the lamp-drying step could be eliminated without any large effects on the sample signal levels (Table 2-2). Both drying and equilibration could now take place in the sample compartment without the use of an infrared lamp to dry the samples. Nitrogen was then compared to argon as the flush gas. The decision to try nitrogen over argon was a financial one. Argon was purchased in tank-form while nitrogen was available in-house as boil-off from a liquid nitrogen reservoir. From the data in Table 2-3, there is no large difference in signal levels if one substitutes nitrogen gas as the flush gas.

Thus, in the modified procedure, after spotting the samples, the bar is placed into the sample compartment where the samples are allowed to dry for 7 min under a flow of dehumidified nitrogen gas (∿20 L/min). During the drying process, sample phosphorescence signal levels increase over a period of ∿7 min at which time a plateau is reached for ∿2 min (Figure 7). Measurements are made on this plateau.

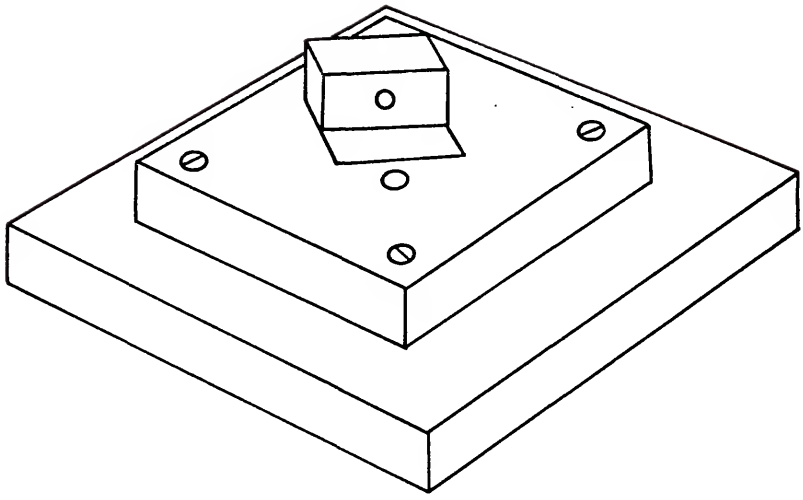


Figure 6. Modified sample compartment lid for use with the RTP-sampling bar in a front-surface flush configuration.

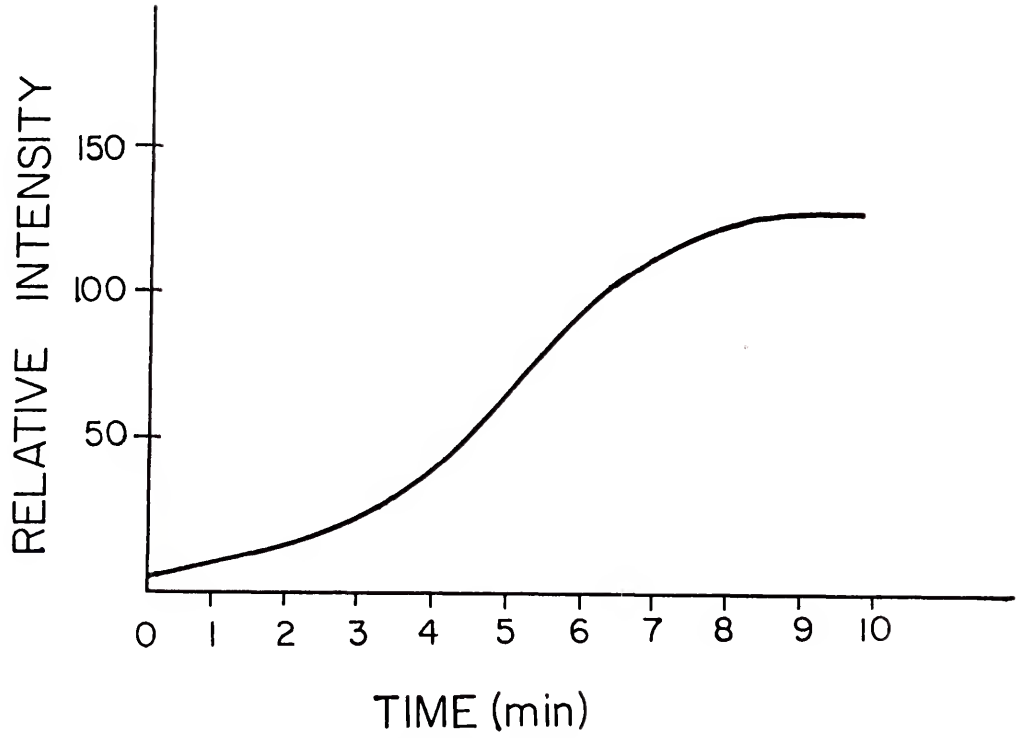


Figure 7. Relative phosphorescence intensity (PABA test solution) versus drying time (front-surface flush configuration).

Table 2-2. Comparison of the Lamp-dry/Back-side Flush Procedure to the Front-surface Dry/Flush Procedure in RTP

Procedure	RPI (A)		Total Time (min) (B)
Lamp-dry/ Back-side Flush	144	138	30
	144	141	
	144	135	
	141	138	
	$\bar{x}$ = 141		
Front-surface Dry/Flush	111	114	10
	114	114	
	108	114	
	111	117	
	$\bar{x}$ = 113		

(A) RPI (relative phosphorescence intensity) using a PABA test solution (50 µg/mL). Argon used as flush gas.

(B) Total time to process 4 samples.

Table 2-3. Comparison of Argon and Nitrogen as a Flush Gas in RTP

Gas	RPI (A)	
Argon	108	111
	111	114
	105	114
	108	114
	$\bar{x}$ = 111	
Nitrogen	120	114
	129	129
	129	111
	120	117
	$\bar{x}$ = 121	

(A) RPI (relative phosphorescence intensity) using a PABA test solution (50 µg/mL). Both gases used in front-surface flush configuration with a 7 min flush time.

Summary

For all practical purposes, the instrumentation, operating conditions and sampling procedure have, up to this point, been optimized. However, the selection of a suitable support material requires further consideration. While filter paper appears to work well, there are literally hundreds of such products on the market. An evaluation of cellulose (used in making filter paper) as a substrate material is discussed in the next chapter.

CHAPTER 3
AN EVALUATION OF CELLULOSE AS A SUBSTRATE MATERIAL FOR ROOM TEMPERATURE PHOSPHORESCENCE

Introduction

The ultimate success of RTP as a method of chemical analysis depends on the selection of a suitable support material. In the past, support materials such as cellulose, silica gel and sodium acetate have been used in RTP measurements of a variety of organic compounds (1-3). Of the three major support materials, cellulose appears to offer a considerable advantage in that there exists a wide-range of specialty papers with varying characteristics. The major disadvantage of cellulose is the presence of a broadband phosphorescence background (at ∿400-600 nm) (29-31). At this laboratory, several filter papers have been evaluated and physical and chemical treatments to minimize the phosphorescence background of the papers have been unsuccessful (32,33). In the present study, several cellulose pulps are evaluated as support materials to determine if there exists a successful combination of physical/chemical characteristics of cellulose pulp for RTP; treated filter paper is also evaluated to find a possible source of the background phosphorescence. For the reader's convenience, a glossary

(34) (Appendix 1) is included to explain terms (used in this chapter) common to the pulp and paper industry but otherwise unfamiliar to the layperson.

Experimental

Reagents and Materials

The following companies kindly provided the respective materials: Buckeye Cellulose (grade 503 cotton linters pulp); ITT Rayonier (Cellunier-P wood pulp); Southern Cellulose (grades 270, 277 and 282-R cotton linters pulps); Eaton-Dikeman (613 and 631 filter papers); Schleicher & Schuell (S & S 903 filter paper-lots W01, W02, W12, W92, W93, W94). Diethylenetriaminepentaacetic acid (DTPA) and p-aminobenzoic acid (PABA) were purchased from Sigma Chemical Co. and were used without further purification. Sodium hydroxide, periodic acid, ether, dioxane and potassium iodide were used as received from Mallinckrodt, Inc. Absolute ethanol, from U.S. Industrial Chemicals Co., was used to prepare solutions with purified water obtained from a Barnsted NANOpure system.

Procedure

Following the selected treatments (Table 3-1), the sheets of S & S 903 filter paper were allowed to air-dry in a photographic darkroom for 12 hr. Next, 0.25-in diameter filter paper discs obtained with a standard office paper punch were placed under the cover plate of the bar, and the plate was screwed down onto the discs. Using a "Micro/pettor" (Scientific Manufacturing Industries), 5 µL

of blank (1 M KI, 1 M NaOH in 50/50 v/v ethanol/water) or 5 µL of analyte (50 µg/mL PABA in 50/50 v/v ethanol/water) were spotted onto the paper discs. The bar was then placed in the sample compartment where the discs were allowed to dry for 7 min under a flow of dehumidified nitrogen gas. For each evaluation, 8 or 16 independent measurements were made for both blank (λex/λem, 320/475 nm) and PABA (λex/λem, 296/432 nm). The blank excitation and emission wavelengths were chosen to give the largest signals which are similar to the substrate background phosphorescence (31). Blank signals were ∿40% lower at the wavelengths set for PABA.

For the lot-analysis (Table 3-4) of S & S 903 filter paper, all lots were treated with a DTPA soak for 24 hr, rinsed for 3 min in water, and allowed to air-dry for 12 hr in a photographic darkroom.

For the handsheet evaluation (Table 3-2), handsheets (basis weight 190±3% g/m^2, thickness 0.5±5% mm) were made from each source of cellulose pulp by two different methods. The first handsheet was made from a sample of each pulp as received from the processing plant. The second handsheet was made from a sample of each pulp that was mechanically beaten for 1 hr.

The sampling procedure for the lot-analysis and handsheet evaluation followed the same sequence as previously described.

Results and Discussion

Background on Papermaking

In order for one to understand the results obtained in this study, one should have a fundamental knowledge of the papermaking process and more specifically, a knowledge of the types of cellulose used in making filter paper products. This fundamental knowledge will allow a more critical evaluation of the substrates used in RTP. However, a detailed description of the papermaking process is beyond the scope of this chapter, and so interested readers are urged to consider reading some of the classical books on pulp and paper technology (35-38). In addition, more recent texts give updated information on testing procedures used in paper analysis (39-41).

Generally speaking, purified cellulose can be obtained from two major sources -- cotton and wood. Wood is comprised mainly of cellulose (~55%) and to lesser extents of hemicelluloses and lignin (the fractions of each dependent upon the type of wood). Cellulose is essential for papermaking while the hemicelluloses can be beneficial in making different types of paper; lignin, on the other hand, is undesirable due to its effects on sheet formation and is removed during chemical pulping and bleaching. The final percentage of cellulose found in wood pulps can reach up to 90% for specific pulping methods. Cotton fibers are approximately 95% cellulose with minor amounts of waxes

and pectins and very little lignin. Chemical processing of cotton fibers can give yields of 99+% cellulose.

Two broad classes of woods of commercial value to the pulp and paper industry are softwoods (pines, spruces, firs and cedars) and hardwoods (oaks, gums, beeches, birches and eucalyptuses). The major types of fibers found in softwood trees are the springwood fibers and the summerwood fibers. Paper sheets made with a high percentage of springwood fibers (flexible fibers with flat surfaces that pack more closely together) are relatively stronger, more dense and less porous. The best source of springwood fiber is central Canadian softwood pulp (~75% springwood). Hardwoods have much shorter fibers than softwoods and as a result do not bond well (sheet has low tensile strength), but they do promote good sheet formation (fewer gaps in sheet).

The two types of fibers found on most varieties of cottonseeds are the lint (staple) fibers and the linters. Lint fibers are used mostly in the textile industry, while cotton linters are processed into pulp for papermaking or for chemical derivatization products. Comparisons of the two cotton fibers show distinct differences. The lint fibers can grow up to 30 mm in length with cell wall thicknesses of up to 3 μm; cotton linters average 4 mm in length with cell wall thicknesses of up to 10 μm. Lint fibers with thin cell walls and wide lumens collapse when dried and thus add strength and density to a sheet of paper.

Cotton linters, on the other hand, with thick cell walls remain round on drying and impart bulk and porosity to paper. Because of these characteristics, cotton linters pulp is used extensively in filter paper applications (42).

Diagnostical Studies on Cellulose Products

This study began with a selection of cellulose products with properties complementing the theories (2) of hydrogen bonding and/or electrostatic interactions giving rise to rigid adsorption of organic molecules onto surfaces of solid supports for which RTP of the compounds could then be observed. The selection included an extremely "pure" wood pulp (Cellunier-P), an equally "pure" cotton linters pulp (Buckeye Cellulose 503), additional cotton linters pulps (Southern Cellulose 270, 277 and 282-R) with tailored properties and several "good" commercially available filter papers (Eaton-Dikeman 613 and 631 and S & S 903).

Phosphorescence background. The first set of experiments (Table 3-1) was set up to evaluate the phosphorescence background of cellulose. Contrary to popular belief, the vast majority of filter paper companies marketing products in the clinical fields do not add special (luminescent) chemicals (optical whiteners, sizing agents, etc.) to their pulps. The desired characteristics of the papers are met through chemically treating (caustic cooking/extracting and inorganic bleaching followed by extensive washings with purified water) and physical manipulation (refining, beating, etc.) of the pulps (43). Thus, filter papers are

relatively pure with the content of cellulose approaching 100%. However, a reasonable question concerns the magnitude of the phosphorescence of cellulose when "bone dry" (31). Lloyd and Miller (44) have observed that highly purified cotton does not phosphoresce and that the phosphorescence may be attributed to trace contaminants absorbed on the cotton. Similarly, Atalla and Nagel (45) have observed that trace amounts of transition metals incorporated into the crystalline domains of cellulose fibers may be responsible for laser-induced fluorescence in cellulose. More recently, Timell (46), Huwyler and coworkers (47,48), Delmer and coworkers (49), Waterkeyn (50) and others (51-55) have pointed out that filter paper cannot be "pure" cellulose because cotton fibers contain trace amounts of hemicelluloses and lignin. These hemicellulosic fractions (mainly β-1,3-glucans) increase at the onset of secondary wall formation and contain species that luminesce.

The various soaking treatments (results in Table 3-1) were structured to test the presence of trace metals and hemicelluloses and/or lignin in cellulose pulp. The chelating agent DTPA was used in an attempt to remove trace amounts of transition metals that were possibly involved in contributing to the phosphorescence background in the filter paper. While the background phosphorescence of the blank was reduced slightly, the analyte signal improved significantly. This treatment gave no improvement for blank levels over past procedures (33). However, owing

Table 3-1. Summary and Results of Treatment of S & S 903 Filter Paper

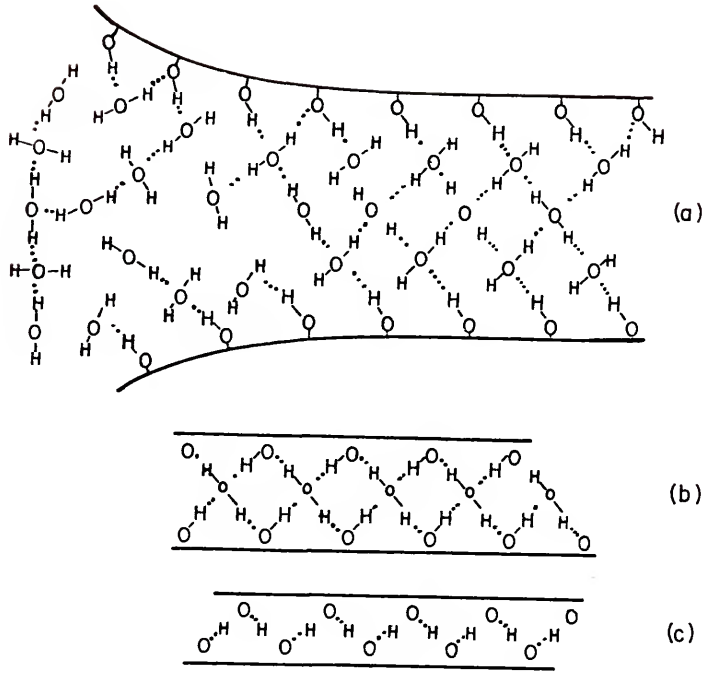
Nature of Treatment	Time of Treatment (hr)	Change in Mean Relative Signal (A)	
		Blank	PABA
Dioxane/Water (50/50) Soak	48	-2	+1
DTPA/Water (saturated) Soak	48	-1	+2
Ether Soak	24	-1	0
Boiling water Soak	0.5	0	0
Eimac lamp (250-W) Bleach	12	0	-2
Sunlight Bleach	24	+2	-1
Sodium hydroxide (3.5 M, 5°C) Soak	24	0	-1
Periodic acid (0.1 M) Soak	12	*	*

(A) Mean given for 8 determinations; change in signal relative to untreated S & S 903 (1980 W94 stock)
-2 signal decreased by ∿100%
-1 signal decreased by ∿50%
0 no change in signal
+1 signal increased by ∿50%
+2 signal increased by ∿100%
* no net signal observed

to possible interactions of DTPA's multi-charged structure with PABA and to the gaps in the paper structure being filled (see Figure 8), this treatment showed a greater effect on the analyte phosphorescence signal. Unfortunately, the soaking with DTPA gave no clues as to whether trace metals were involved in the phosphorescence background of cellulose.

After Millson's work (29) an Eimac lamp (250 W-xenon arc) and sunlight were used to try to bleach out "impurities" in the filter paper. This prolonged illumination was actually detrimental to the paper as analyte signals decreased due to possible disruption of the surface structure.

The remaining treatments were used to evaluate the presence of extractable hemicelluloses (and/or lignin) in cellulose pulp. While none of the treatments unequivocally confirmed the presence of such materials, the dioxane soaking indicated (by a large decrease in background phosphorescence) that an extractable hemicellulosic (and/or lignin) fraction may have been present. Periodic acid oxidation (53) almost destroyed surface hydroxyl groups (to form aldehyde groups), and no net analyte signal or background was observed. Thus, while hemicelluloses (and/or lignin) appear to be responsible for the background phosphorescence, complete removal of these groups apparently disrupts the surface structure, and analyte phosphorescence does not occur (52).



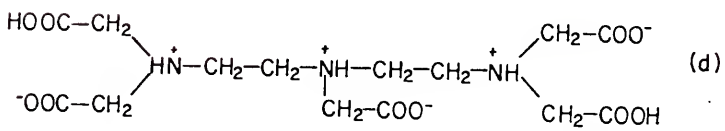


Figure 8. Simulation of hydrogen bonding between two cellulose molecules:

a. Loosely through water molecules.
b. Tightly through a monolayer of water molecules
c. Directly
d. Loosely through the transposition of DTPA into (a).

Fibrillation study. In the fibrillation study (Table 3-2), two handsheets (one more highly fibrillated than the other) were made from unbeaten and beaten pulps. Beating cellulose pulps over a period of time decreases the average fiber length and increases the average exposed surface area. Therefore, with more hydroxyl groups exposed on the surface, more complete adsorption of organic molecules on the support material takes place and an enhancement in phosphorescence should be seen. But, at the same time, fibrillation increases the exposure of the hemicellulosic material located within the inner matrix of the cellulose fibers. As a result, a larger phosphorescence background should be seen. In fact, the results (Table 3-2) obtained for Cellunier-P, Buckeye Cellulose 503, Southern Cellulose 270, 277 and 282-R appear to reflect on these generalizations. While the degrees of fibrillation have not been confirmed for Cellunier-P and Buckeye Cellulose 503, one would expect Buckeye Cellulose 503 cellulose pulp to be more fibrillated than the Cellunier-P pulp. The trend for the three Southern Cellulose pulps appear to follow in order with grade 270 being less fibrillated than grade 277 which in turn is less fibrillated than grade 282-R.

Filter paper comparison. Eaton-Dikeman filter papers 613 and 631 were compared to S & S 903 filter paper (see Table 3-3) to determine whether paper porosity affects the interaction of the cellulose surface groups with analyte molecules. An optimal filter paper should allow the bulk of

Table 3-2. Results of Handsheet Evaluations

Sample[A]	Mean Relative Signal[B]				S_A/S_B[C]	
	Blank		PABA			
	H_1	H_2	H_1	H_2	H_1	H_2
ITT Rayonier Cellunier-P	4.0	5.0	120	180	30	36
Buckeye Cellulose 503	4.5	4.5	180	235	40	52
Southern Cellulose 270	4.8	5.3	190	225	40	42
Southern Cellulose 277	5.7	7.5	310	340	54	45
Southern Cellulose 282-R	8.3	11.0	300	370	36	34

(A) H_1 - 1st handsheet - no pre-treatment of pulp.
H_2 - 2nd handsheet - pulp beaten for 1 hour prior to handsheet formation.

(B) Mean relative signal calculated from 8 determinations.

(C) Ratio of mean relative signal of PABA (S_A) to mean relative signal of blank (S_B).

Table 3-3. Results of Filter Paper Comparisons

Filter Paper[A]	Mean Relative Signal[B]		S_A/S_B
	Blank	PABA	
S & S 903	10.7	150	14
S & S 903 (DTPA-treated)	6.8	365	54
Eaton-Dikeman 613	6.4	135 (225)	21 (35)
Eaton-Dikeman 631	6.6	125 (208)	19 (32)

(A) S & S 903 (basis weight ~190 g/m^2, thickness ~0.45 mm)
Eaton-Dikeman 613 and 631 (basis weight ~70 g/m^2, thickness ~0.2 mm)
5 µL volumes used with S & S paper
3 µL volumes used with Eaton-Dikeman papers
Numbers in parentheses represent signals (multiplied by 5/3) that would be obtained using 5 µL volumes assuming linearity of determination.

(B) Mean relative signal calculated from 8 determinations.

the analyte to remain upon the surface to accommodate effective adsorption and/or interaction. Thus, "slow" filter papers are the best choices. While the Eaton-Dikeman 613 and 631 papers gave larger analyte signals than the untreated S & S 903 paper, the DTPA-treated S & S 903 paper gave substantially larger signals. The discussion of DTPA filling in the gaps of S & S 903 now becomes apparent (by the larger signals) as the analyte molecules are "trapped" in the DTPA-cellulose matrix (56).

Filter-paper lots comparison. The various lots (see Table 3-4) of S & S 903 filter paper are consistent in quality for use in RTP applications. On a statistical basis (57) (Duncan's multiple range procedure with $\alpha=0.01$, using a completely randomized design with 105 degrees of freedom and mean square values of 0.058 and 130 for blank and analyte signals, respectively), the W94 (1980) and W93 lots are significantly different (for analyte signals) from the other lots of paper. For blank signals, the W94 (1980) and W12 lots are significantly different from the other lots of paper.

Conclusion

In view of the results obtained in this study, it is clear that cellulose-based support materials cover a wide range of products; nevertheless, for RTP applications, it is practical to assume that the difference in performance between the poorest paper and the best paper is considerably less than an order of magnitude. Thus, researchers

Table 3-4. Results of Lot-Analysis of DTPA-treated S & S 903 Filter Papers

Lot[A]	Blank		PABA		S_A/S_B
	Mean Relative Signal	CV(%)	Mean Relative Signal	CV(%)	
W94 (1980)	6.3	3.7	360	3.2	57
W94 (1981)	5.8	4.4	405	2.2	70
W93	5.4	4.3	423	3.5	78
W92	5.2	3.4	408	2.3	78
W12	6.3	3.6	399	2.7	63
W02	5.3	4.7	407	3.3	77
W01	5.2	3.5	400	2.4	77

(A) Mean relative signals and coefficient of variation (CV,%) calculated from 16 measurements of blank and PABA. S_A (mean relative signal of PABA); S_B (mean relative signal of blank).

evaluating RTP for analytical studies should select support materials giving the highest signal-to-noise ratio. If one chooses to use filter papers (out of convenience of availability), then a pre-treatment is recommended to enhance its adsorption characteristics. If indeed the background phosphorescence can not be reduced in cellulose-based products (and it seems unlikely it can be significantly reduced), time-resolved phosphorimetry may effectively correct for such interferences. As a matter of choice, all subsequent studies in RTP were made using DTPA-treated S & S 903 filter paper as the substrate material.

Note

[1]The author is indebted to Mr. Whitten Bell (Buckeye Cellulose), to Mr. Fred Mathis (Southern Cellulose) and to Mr. Harvey Wilson (ITT Rayonier) for samples of cotton linters and wood pulps and for technical information and advice. Gratitude is extended to Mr. Steve Ritchie and to Mr. Bob Edwards (ITT Rayonier, Fernandina Beach, FL) for samples of wood pulps and for the informative tour of the pulp mill. Special thanks is given to Mr. Bradd Levine (Eaton-Dikeman) and to Mr. Lothar Jeschke (Schleicher & Schuell) for samples of filter paper. The author is also indebted to Mr. John Baumgardner (Eaton-Dikeman) for making the handsheets of paper, to Dr. Rajai Atalla (Institute of Paper Chemistry, Appleton, WI), and especially to Dr. Ralph Berni (USDA-Southern Regional Research Center, New Orleans, LA) for paper analysis and for helpful suggestions. An additional note of appreciation is extended to all others in the pulp and paper industry for their cooperation on this project.

CHAPTER 4
BIOCHEMICAL AND DRUG ANALYSIS BY ROOM TEMPERATURE PHOSPHORESCENCE

Introduction

Recent studies in RTP have shown that a wide range of organic molecules exhibit phosphorescence at room temperature when adsorbed on a variety of suitable support materials (2). Several studies have led to developments whereby RTP can now be applied to solving chemical problems and can be used to provide additional information on the phenomenon of phosphorescence of organic molecules. Compounds exhibiting RTP are usually grouped in classes of ionic or polar organic molecules; under special circumstances, certain nonpolar organic molecules (polynuclear aromatic hydrocarbons) exhibit RTP. The largest group of compounds exhibiting RTP is the azines, which includes quinolines, isoquinolines, quinoxalines, quinazolines, indoles, indazoles, benztriazoles, benzimidazoles and related benzyl-derivatives of these classes. Other groups of compounds exhibiting RTP include purines, the related xanthines, benzoic acid derivatives and related species.

While documentation is available for those compounds exhibiting RTP in the aforementioned groups, there is much overlap in the area and very little mention is given to

those compounds which do not phosphoresce or which phosphoresce weakly and therefore can not be used in analytical studies. Furthermore, most documentation describing RTP as a useful analytical technique goes unsupported in the literature. Only in remote cases does one find the application of RTP to real-sample analysis (58-60).

In this project, groups of molecules were studied to find trends in phosphorescence among related compounds. When a group of compounds was found to exhibit RTP, then appropriate real samples were selected and analyzed by RTP. It was obvious that not all groups of compounds could be studied, so selections were made from the azine group, the purine group and the benzoic acid derivative group. Although care was taken to select only those compounds not previously studied, the selection process was a difficult task; however, overlapping studies were kept to a minimum.

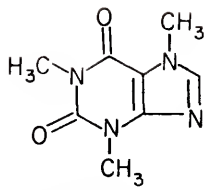
Biochemicals/Drugs

The first group of compounds selected to be studied was the purine/xanthine group. This group was selected for interests in future studies in evaluating the use of RTP in therapeutic drug monitoring (Appendix 2). The major compounds in this group include caffeine, theobromine and theophylline. Caffeine (1,3,7-trimethylxanthine) is a central stimulant and is found in large quantities in coffee, tea and colas. Theobromine (3,7-dimethylxanthine) is used therapeutically as a vasodilator and is principally found in hot chocolate. Theophylline (1,3-dimethylxanthine)

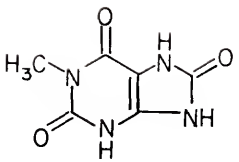
is found in tea and is used therapeutically to treat patients suffering from apnea, asthma and various stages of chronic obstructive pulmonary disease. A summary of these and related compounds and metabolites can be found in Figure 9. The rest of the group will be covered in another section.

The indoles were the second group of compounds studied. Along with several biologically-important compounds, this group includes 5-hydroxyindoleacetic acid, 5- hydroxytryptophan, indole, indoleacetic acid, indole carboxylic acid, indomethacin, melatonin, serotonin and tryptophan. Serotonin serves as a chemical messenger; the presence of high levels of this compound in serum or urine indicates active stages of a malignant carcinoid tumor. Compounds related to serotonin are 5-hydroxytryptophan, a precursor, and 5-hydroxyindoleacetic acid, a degradation product. Indole is used in the perfume industry. Indoleacetic acid is a plant growth regulator. Indomethacin, because of its anti-inflammatory and analgesic effects, is useful for treating certain types of arthritis. Melatonin is a skin pigment factor. Tryptophan is an essential amino acid because it is not synthesized by the human body. Figures 10 and 11 illustrate the structural similarity between these compounds.

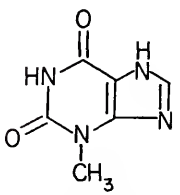
The third group studied contains a benzene ring as the central structural element. Included in this group are a variety of compounds shown in Figures 12-16. Benzoic



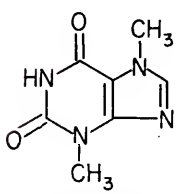
Caffeine
1,3,7-trimethylxanthine



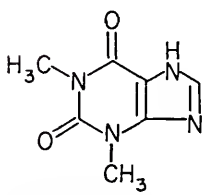
1-methyluric acid



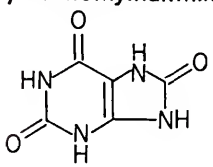
3-methylxanthine



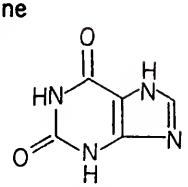
Theobromine
3,7-dimethylxanthine



Theophylline
1,3-dimethylxanthine

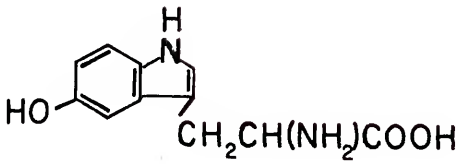


Uric acid

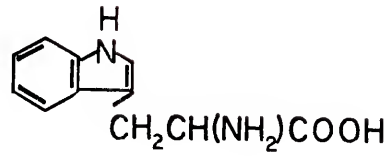


Xanthine

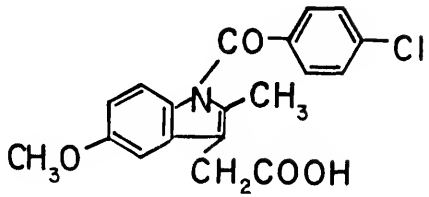
Figure 9. Chemical structures of several xanthines and uric acids.



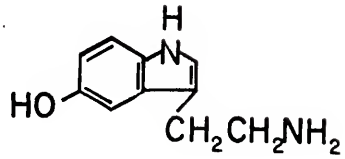
5-HYDROXYTRYPTOPHAN



TRYPTOPHAN

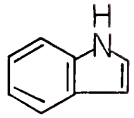


INDOMETHACIN

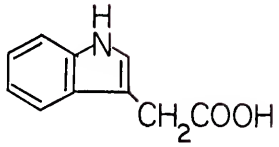


SEROTONIN

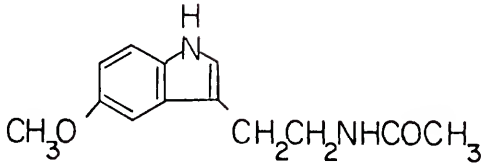
Figure 10. Chemical structures of several indole-related compounds.



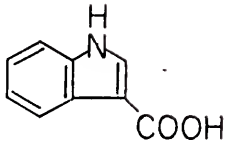
INDOLE



INDOLEACETIC ACID



MELATONIN



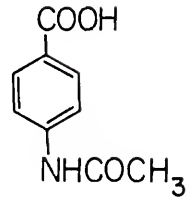
INDOLE CARBOXYLIC ACID

Figure 11. Chemical structures of indole and several indole derivatives.

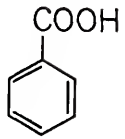
acid, methylparaben, propylparaben and vanillin function as pharmaceutic aids. Two compounds, p-aminobenzoic acid and p-aminohippuric acid, serve as diagnostic aids in studying pancreatic and renal function, respectively. p-Aminobenzoic acid is also commonly used as a sunscreen agent. p-Acetamidobenzoic acid is sometimes used as an antidepressant drug. p-Hydroxybenzoic acid functions as a reactive intermediate for organic dye synthesis. Sulfosalicylic acid is used as an analytical reagent for the determination of protein (albumin) in urine. Acetylsalicylic acid and salicylamide, often used in combination, are common analgesics. Sulfacetamide functions as an antimicrobial agent while p-aminosalicylic acid is an antitubercular agent. Salicylic acid, a keratolytic, is an active ingredient in medicated cleansers used for the treatment of acne. Probenecid functions as an uricosuric. Lidocaine and procaine are common local anesthetics.

A group of miscellaneous compounds was also studied. Chloroquine and primaquine, two antimalarial drugs, and naphazoline, a vasoconstrictor, are shown in Figure 17. Propranolol and dibucaine, a heart drug and a local anesthetic, respectively, are shown in Figure 18. A group of antihypertensive agents (chlorthiazide, diazoxide, hydrochlorthiazide, reserpine and trichlormethiazide) and an antibacterial agent (tetracycline) are shown in Figures 19 and 20.

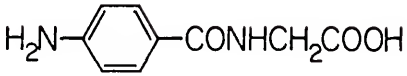
Figure 12. Chemical structures of benzoic acid and several benzoic acid derivatives.



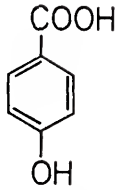
p-ACETAMIDOBENZOIC ACID



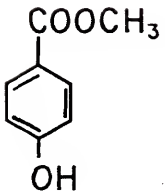
BENZOIC ACID



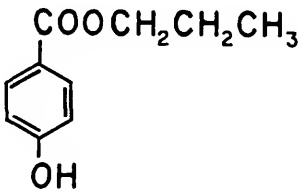
p-AMINOHIPPURIC ACID



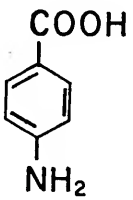
p-HYDROXYBENZOIC ACID



METHYLPARABEN



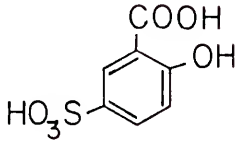
PROPYLPARABEN



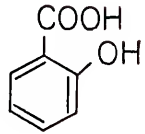
p-AMINOBENZOIC ACID

Figure 13. Chemical structures of several benzoic acid-related compounds.

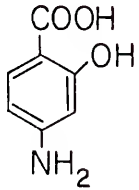
Figure 14. Chemical structures of salicylic acid and related derivatives.



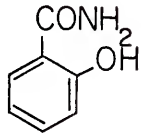
SULFOSALICYLIC ACID



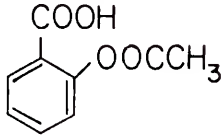
SALICYLIC ACID



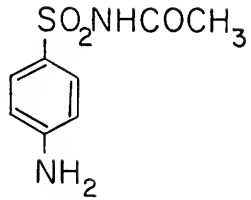
p-AMINOSALICYLIC ACID



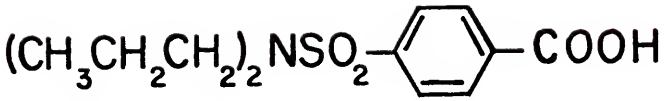
SALICYLAMIDE



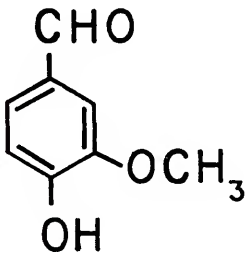
ACETYLSALICYLIC ACID



SULFACETAMIDE

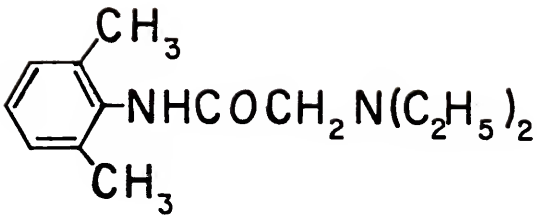


PROBENECID



VANILLIN

Figure 15. Chemical structures of a uricosuric (top) and a pharmaceutic aid(bottom).

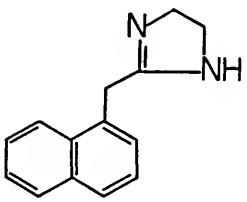


LIDOCAINE

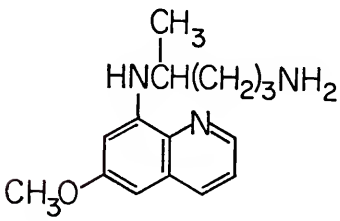
PROCAINE

Figure 16. Chemical structures of two common local anesthetics.

CHLOROQUINE

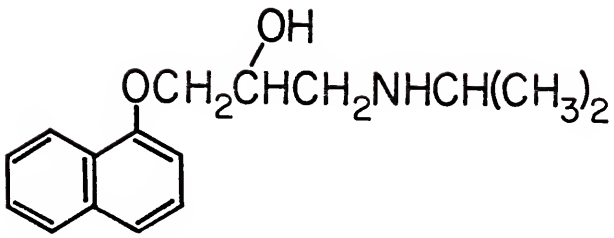


NAPHAZOLINE

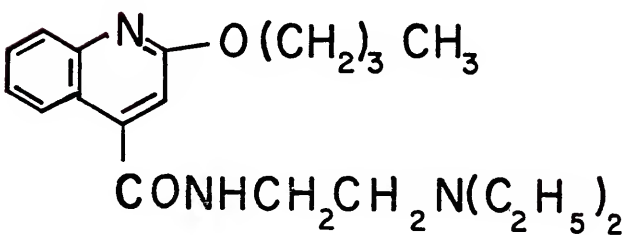


PRIMAQUINE

Figure 17. Chemical structures of two antimalarial drugs(top and bottom) and a vasoconstrictor(middle).

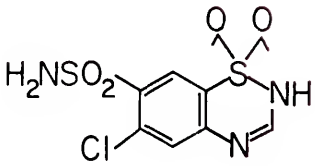


PROPRANOLOL

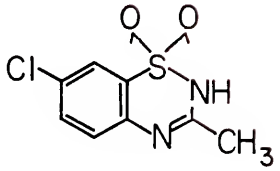


DIBUCAINE

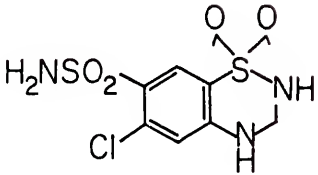
Figure 18. Chemical structures of a heart drug (top) and a local anesthetic (bottom).



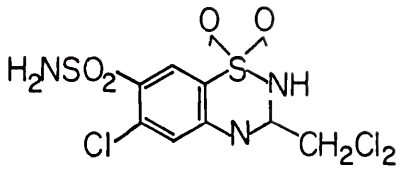
CHLORTHIAZIDE



DIAZOXIDE

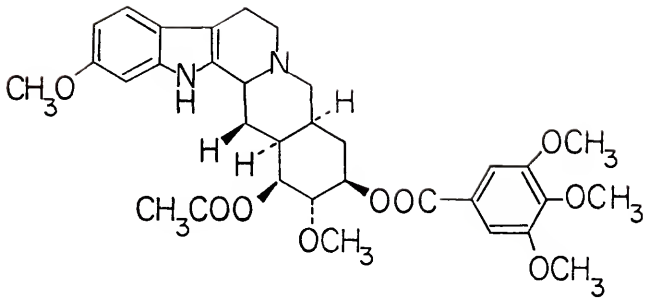


HYDROCHLORTHIAZIDE

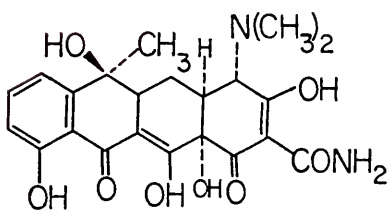


TRICHLORMETHIAZIDE

Figure 19. Chemical structures of several antihypertensive agents.

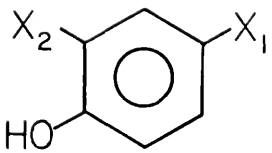


RESERPINE



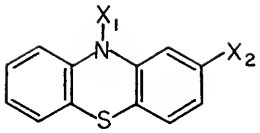
TETRACYCLINE

Figure 20. Chemical structures of an antihypertensive drug(top) and an antibacterial agent(bottom).



	X_1	X_2
3,4-Dihydroxymandelic acid	CHOHCOOH	OH
Dopa	$CH_2CH(NH_2)COOH$	OH
Dopamine	$CH_2CH_2NH_2$	OH
Epinephrine	$CHOHCH_2NHCH_3$	OH
Homogentisic acid	CH_2COOH	OH
Homovanillic acid	CH_2COOH	OCH_3
Isoproterenol	$CHOHCH_2NHCH(CH_3)_2$	OH
Metanephrine	$CHOHCH_2NHCH_3$	OCH_3
3-Methoxytyramine	$CH_2CH_2NH_2$	OCH_3
Norepinephrine	$CHOHCH_2NH_2$	OH
Tyrosine	CH_2CHNH_2COOH	—
Vanillylmandelic acid	CHOHCOOH	OCH_3
Normetanephrine	$CHOHCH_2NH_2$	OCH_3

Figure 21. Chemical structures of a series of catechol-related compounds.



	X_1	X_2
Acetophenazine	$(CH_2)_2CH_2-N\bigcirc N-CH_2CH_2OH$	$COCH_3$
Chlorpromazine	$(CH_2)_2CH_2N(CH_3)_2$	Cl
Fluphenazine	$(CH_2)_2CH_2-N\bigcirc N-CH_2CH_2OH$	CF_3
Perphenazine	$(CH_2)_2CH_2-N\bigcirc N-CH_2CH_2OH$	Cl
Prochlorperazine	$(CH_2)_2CH_2-N\bigcirc N-CH_3$	Cl
Promazine	$(CH_2)_2CH_2N(CH_3)_2$	—
Promethazine	$CH_2\overset{CH_3}{C}HN(CH_3)_2$	—
Thiopropazate	$(CH_2)_2CH_2-N\bigcirc N-CH_2CH_2OOCCH_3$	Cl
Thioridazine	$CH_2CH_2-\overset{CH_3}{N}\bigcirc$	SCH_3
Trifluoperazine	$(CH_2)_2CH_2-N\bigcirc N-CH_3$	CF_3
Triflupromazine	$(CH_2)_2CH_2N(CH_3)_2$	CF_3
Trimeprazine	$CH_2\overset{CH_3}{C}HCH_2N(CH_3)_2$	—

Figure 22. Chemical structures of a series of phenothiazines.

Two other groups of compounds (Figures 21 and 22), studied quite-extensively by LTP (61,62), were studied here at room temperature. However, with just a few exceptions, these compounds did not exhibit RTP under the specific test conditions used in these studies. These results will be discussed in a later section.

Pharmaceutical Formulations

Upon completion of the RTP studies with the diverse selection of compounds, optimum conditions were established for which each compound or groups of compounds could be analyzed by RTP (see Table 4-3). Next, samples of compounds were considered for real-sample applications. For practical considerations, several drugs and their respective pharmaceutical formulations or over-the-counter preparations were selected as working systems.

Oral administration of drug substances, via solid dosage forms (e.g., tablets and capsules), is the most frequent route used in distributing drugs in biological systems. Large-scale production of such substances requires the presence of a variety of other materials to complement the active ingredient. Other additives may also be used in formulations to enhance physical appearance, improve stability or aid in drug distribution. These supposedly inert additives must be considered when assay procedures are established for quality control protocol of manufactured formulations.

In recent years, generic brands of drugs have attained widespread use in response to consumer groups' participation in regulatory agencies (63). Generic brands of drugs offer practitioners a wide selection of products for which therapeutic dosage regimens can be determined for individual patients. Specific formulations are used to achieve the desired pharmacodynamic/pharmacokinetic responses in patients. Because generic manufacturers often use a diverse group of diluents, binders, lubricants, coloring and flavoring agents, preservatives, etc., in their formulations, a specific procedure is needed for the quantitation of active ingredients in the formulations.

Current U.S.P. procedures (64) for analyzing a variety of drugs substances in pharmaceutical formulations are rather cumbersome and as a result, many pharmaceutical manufacturers are evaluating the use of high performance liquid chromatography (HPLC) and luminescence methods of analysis for quality control procedures.

In this section, RTP is used for the analysis of a variety of drugs in pharmaceutical formulations and over-the-counter preparations (see Table 4-2). The selection of formulations and preparations to be analyzed was made by consulting the American Drug Index (65). Under the various generic names of drugs, a wide range of preparations was selected to represent different matrices within which the drugs were contained. The selections were made to illustrate the selectivity advantage of RTP as a method of analysis of complex mixtures.

Experimental

Reagents and Materials

The analytical reagents used in these studies were previously described in Chapter 3. All other chemicals are listed in Table 4-1. Commercial preparations used in these studies were purchased at a local hospital pharmacy (Shands Teaching Hospital and Clinics, Gainesville, FL) and are listed in Table 4-2.

Sample Preparation

Biochemicals/drugs. For the RTP studies of the compounds listed in Table 4-1, standard stock solutions (200-400 µg/mL) were prepared by dissolving accurately weighed portions of the compounds in an ethanolic solution (50/50 v/v ethanol/water). Next, the samples were measured in four steps to determine the optimum solvent system to use for RTP studies. The four basic solvent systems were (1) ethanolic (2) ethanolic + 1 M KI (3) ethanolic + 1 M NaOH (4) ethanolic + both 1 M KI and 1 M NaOH. The addition of 1 M KI served as a heavy atom perturber. The 1 M NaOH addition served to "ionize" the molecules to allow for a more effective interaction (electrostatic or hydrogen bonding) of the molecules with the substrate material. An optimal solvent system was selected by comparing phosphorescence intensities (using a 100 µg/mL test solution) obtained using the various solvent systems. Where applicable, by using solubility data (66), the amount of ethanol in each optimum solvent system was reduced to minimize the effects

Table 4-1. Sources of Chemicals Used in RTP Studies

Compound	Source
p-Acetamidobenzoic acid	(A)
Acetaminophen	(A)
Acetophenazine	(B)
Acetylsalicylic acid	(A)
Allopurinol	(A)
Alloxan	(A)
p-Aminobenzoic acid	(A)
p-Aminohippuric acid	(A)
Aminophylline	(A)
Aminosalicylic acid	(A)
Amitriptyline	(B)
Anisindione	(B)
Atropine	(A)
Azathioprine	(A)
Benzoic acid	(A)
Caffeine	(A)
Calmagite	(A)
Chloramphenicol	(C)
6-Chloropurine	(A)
Chloroquine	(A)
8-Chlorotheophylline	(A)
Chlorpheniramine	(B)
Chlorphentermine	(C)
Chlorpromazine	(D)
Danthron	(E)
Dexbrompheniramine	(B)
Dexchlorpheniramine	(B)
2,6-Diaminopurine	(A)
Diazoxide	(B)
Dibucaine	(F)
Dihydroxymandelic acid	(A)
1,3-Dimethyluric acid	(A)

Table 4-1-continued.

Compound	Source
1,7-Dimethylxanthine	(A)
Diphenhydramine	(A)
Dopa	(A)
Dopamine	(A)
Dyphylline	(A)
Ephedrine	(A)
Epinephrine	(E)
Ethosuximide	(C)
Fluphenazine	(B)
Folic acid	(A)
Folinic acid	(A)
Furosemide	(G)
Gitalin	(B)
Gossypol	(A)
Griseofulvin	(B)
Guaiacol glyceryl ether	(A)
Homogentisic acid	(A)
Homovanillic acid	(A)
Hydrochlorthiazide	(F)
p-Hydroxybenzoic acid	(A)
5-Hydroxyindoleacetic acid	(A)
5-Hydroxytryptophan	(A)
β-Hydroxyethyltheophylline	(A)
Indole	(A)
Indole carboxylic acid	(A)
Indoleacetic acid	(A)
Indomethacin	(A)
Inosine	(A)
Inosinic acid	(A)
Isoproterenol	(A)
Lidocaine	(A)
Mefenamic acid	(C)

Table 4-1-continued.

Compound	Source
Melatonin	(A)
6-Mercaptopurine	(A)
Metanephrine	(A)
Methotrexate	(A)
3-Methoxytyramine	(A)
Methsuximide	(C)
Methylparaben	(A)
1-Methyluric acid	(A)
3-Methyluric acid	(A)
1-Methylxanthine	(A)
3-Methylxanthine	(A)
7-Methylxanthine	(A)
Naphazoline	(F)
Nicotinic acid	(A)
Normetanephrine	(A)
Orphenadrine	(E)
Oxtriphylline	(C)
Oxymetazoline	(B)
Perphenazine	(B)
Phenacetin	(B)
Phenazophridine	(C)
Phenylalanine	(A)
Phenylephrine	(A)
Phenylpropanolamine	(A)
Phenytoin	(C)
Phytic acid	(A)
Prazosin	(H)
Primaquine	(A)
Primidone	(I)
Probenecid	(A)
Procaine	(A)
Prochlorperazine	(D)

Table 4-1-continued.

Compound	Source
Promazine	(J)
Promethazine	(J)
Propranolol	(I)
Propylparaben	(A)
Purine	(A)
Pyrilamine	(A)
Quinidine	(A)
Quinine	(A)
Reserpine	(F)
Riboflavin	(A)
Saccharin	(A)
Salicylamide	(A)
Salicylic acid	(A)
Scopolamine	(A)
Serotonin	(A)
Sulfacetamide	(B)
Sulfosalicylic acid	(A)
Tetracycline	(A)
Theobromine	(A)
Theophylline	(A)
Thiopropazate	(K)
Thioridazine	(L)
Thyroxine	(A)
Tolnaftate	(B)
Trichloromethiazide	(B)
Trifluoperazine	(D)
Triflupromazine	(M)
Trihexyphenidyl	(B)
Trimeprazine	(D)
1,3,7-Trimethyluric acid	(A)
Tripelennamine	(A)
Tryptophan	(A)

Table 4-1-continued.

Compound	Source
Tryosine	(A)
Uracil	(A)
Uric acid	(A)
Vanillylmandelic acid	(A)
Vanillin	(A)
Xanthine	(A)

(A) Sigma
(B) Schering
(C) Warner-Lambert
(D) Smith Kline French
(E) Riker
(F) Ciba-Geigy
(G) United States Pharmacopeia
(H) Pfizer
(I) Ayerst
(J) Wyeth
(K) Searle
(L) Sandoz
(M) Squibb

of ethanol on the cellulose substrate material (53). Table 4-3 lists those compounds (used in this study) that phosphoresce and the solvent systems used to observe the maximum phosphorescence intensities.

Pharmaceutical formulations. In the analysis of the commercial preparations listed in Table 4-2, samples were prepared for assay by dissolution/dilution in/with an

appropriate solvent system. Standard stock solutions were prepared by dissolving accurately weighed portions of standards in an appropriate solvent system. Standard solutions were prepared daily by mixing appropriate volumes of the stock solution with the solvent system. All solutions, when not being analyzed, were stored under suggested conditions to insure stability of the reagents. For analysis of representative samples, 16 or 20 tablets (contents of capsules) were weighed and powdered with a mortar and pestle, and 4 portions (equivalent to 100 μg/mL of active ingredient in a total volume of 5 or 10 mL) were dissolved in the appropriate solvent system. This procedure was repeated for each solid sample. For the analysis of liquid preparations, appropriate volumes (to give final concentrations of 100 μg/mL of active ingredient) of the samples were diluted with the appropriate solvent system. Four different test solutions were prepared for each pharmaceutical formulation or commercial preparation; each test solution contained 100 μg/mL of active ingredient.

Procedure

All bar-RTP measurements were performed as previously described in Chapter 2. Quantitation was achieved by comparing relative phosphorescence intensities of samples to those of standards. For the analyses, linear ranges were established and linear regression analysis was performed on each set of data.

Table 4-2. Sources of Commerical Preparations

Formulation	Active Ingredient	Source
Accurbron™ *	Theophylline	Dow
Aminophyllin	Aminophylline	Searle
Anacin®	Acetylsalicylic acid	Whitehall
Aralen®	Chloroquine	Winthrop
Asbron G® Inlay-tabs®	Theophylline	Dorsey
Athemol®	Theobromine magnesium oleate	Glaxo
Bayer® aspirin	Acetylsalicylic acid	Glenbrook
BC® Powder	Acetylsalicylic acid	BC Remedy
Bronkotabs®	Theophylline	Breon
Bufferin®	Acetylsalicylic acid	Bristol-Myers
Choledyl®	Oxtriphylline	Warner-Lambert
Elixophyllin® Elixir*	Theophylline	Berlex
Empirin®	Acetylsalicylic acid	Burroughs Wellcome
Excedrin™ Extra Strength	Acetylsalicylic acid	Bristol-Myers

Table 4-2—continued.

Formulation	Active Ingredient	Source
Inderal® 20,40,80,1	Propranolol	Ayerst
Inderide® 40/25,80/25	Propranolol/Hydrochlorthiazide	Ayerst
Lufyllin-GG®	Dyphylline	Wallace
NoDoz®	Caffeine	Bristol-Myers
Pabanol™ *	p-Aminobenzoic acid	Elder
Privine®*	Naphazoline	Ciba-Geigy
Quadrinal™	Theophylline calcium salicylate	Knoll
Serpasil®*	Reserpine	Ciba-Geigy
Slo-phyllin® -80 syrup	Theophylline	Dooner
Slo-phyllin® -60 Gyrocaps®	Theophylline	Dooner
Theo-dur®	Theophylline	Key
Vivarin®	Caffeine	J.B. Williams
Wyeth® aspirin	Acetylsalicylic acid	Wyeth

* = Liquid preparations

Table 4-3. Optimum Solvent Systems for Compounds Exhibiting RTP

Compound	Solvent System
p-Acetamidobenzoic acid	(1)
Acetylsalicylic acid	(5)
p-Aminobenzoic acid	(1)
p-Aminohippuric acid	(1)
Aminophylline	(2)
Aminosalicylic acid	(5)
Azathioprine	(4)
Benzoic acid	(4)*
Caffeine	(2)
6-Chloropurine	(4)*
Chloroquine	(1)
2,6-Diaminopurine	(4)
Diazoxide	(4)*
Dibucaine	(1)
1,7-Dimethylxanthine	(2)
Dyphylline	(2)
Folinic acid	(3)
Homogentisic acid	(4)*
Hydrochlorthiazide	(4)*
p-Hydroxybenzoic acid	(1)
β-Hydroxyethyltheophylline	(2)
5-Hydroxyindoleacetic acid	(3)
5-Hydroxytryptophan	(2)
Indole	(3)
Indole carboxylic acid	(3)
Indoleacetic acid	(3)
Indomethacin	(3)*
Melatonin	(3)
6-Mercaptopurine	(4)*
Methylparaben	(3)
1-Methylxanthine	(2)

Table 4-3—continued.

Compound	Solvent System
3-Methylxanthine	(2)
7-Methylxanthine	(2)
Naphazoline	(2)
Oxtriphylline	(2)
Prazosin	(3)*
Probenecid	(4)
Procaine	(3)
Propranolol	(3)@
Propylparaben	(3)
Reserpine	(4)
Salicylamide	(5)
Salicylic acid	(5)
Serotonin	(3)
Sulfacetamide	(5)
Sulfosalicylic acid	(5)
Tetracycline	(4)
Theobromine	(2)
Theophylline	(2)
Trichloromethiazide	(4)*
Tryptophan	(2)
Tyrosine	(2)
Vanillin	(4)
Xanthine	(2)

(1) water

(2) water + 1 M KI

(3) ethanolic + 1 M KI

(4) ethanolic + both 1 M KI and 1 M NaOH

(5) 2/98 v/v ethanol/water + both 1 M KI and 1 M NaOH

* Phosphorescence observed but not analytically useful; i.e., signal levels <10 x blank level.

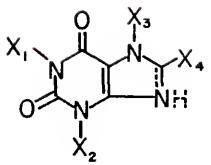
@ 2 M KI

Results and Discussion

Biochemical/Drug Survey

Xanthines. For the biochemical/drug survey, the two major groups studied were the xanthine group and the benzene-based group. Selected compounds in the xanthine group are summarized in Figure 23. For the series of compounds, the phosphorescence intensities follow the trend: dyphylline > caffeine > theobromine > 1,7-dimethylxanthine > theophylline ≅ 7-methylxanthine > 3-methylxanthine > 1-methylxanthine ≅ xanthine. In general, when a substituent other than hydrogen occupies position X_4, no phosphorescence is observed. For the uric acid series, where there is a double-bonded oxygen atom at position X_4, no double bond (dashed line in Figure 23) exists and a hydrogen atom (dashed 'H') is present. β-Hydroxyethyltheophylline, a theophylline derivative with a hydroxyethyl group at position X_3, exhibits the most intense RTP of the xanthine series. Aminophylline and oxtriphylline, ethylene-diamine and choline salts of theophylline, respectively, exhibit RTP characteristic of the theophylline base.

For the xanthine structure, group substitution affects phosphorescence intensity. Group substitution appears to be most effective at position X_3; substitution at position X_2 appears to be more effective than at position X_1. Thus, a methyl group at positions X_1, X_2 or X_3 ($X_1 < X_2 < X_3$ in terms of effectiveness) gives an enhancement in phosphorescence when compared to the phosphorescence obtained when



	X_1	X_2	X_3	X_4
Caffeine	CH_3	CH_3	CH_3	—
8-Chlorotheophylline	CH_3	CH_3	H	Cl
1,3-Dimethyluric acid	CH_3	CH_3	H	=O
1,7-Dimethylxanthine	CH_3	H	CH_3	—
Dyphylline	CH_3	CH_3	$CH_2CH(OH)CH_2OH$	—
1-Methyluric acid	CH_3	H	H	=O
3-Methyluric acid	H	CH_3	H	=O
1-Methylxanthine	CH_3	H	H	—
3-Methylxanthine	H	CH_3	H	—
7-Methylxanthine	H	H	CH_3	—
Theobromine	H	CH_3	CH_3	—
Theophylline	CH_3	CH_3	H	—
1,3,7-Trimethyluric acid	CH_3	CH_3	CH_3	=O
Uric acid	H	H	H	=O
Xanthine	H	H	H	—

Figure 23. Chemical structures of a series of compounds in the xanthine group. A hydrogen atom occupies X_4 unless otherwise noted.

a hydrogen atom occupies either of the positions. At this time, there are no plausible explanations for the varied effects of methyl group substitution at X_1, X_2 or X_3 on the observed RTP intensities. In theory, the presence of methyl groups (electron-donating groups) in lieu of hydrogen atoms at these positions (X_1, X_2, X_3) may contribute to enhanced resonance stabilization of the molecules. This resonance stabilization may ultimately affect the stability of the triplet states of the molecules which in turn could explain the observed trends in phosphorescence intensity.

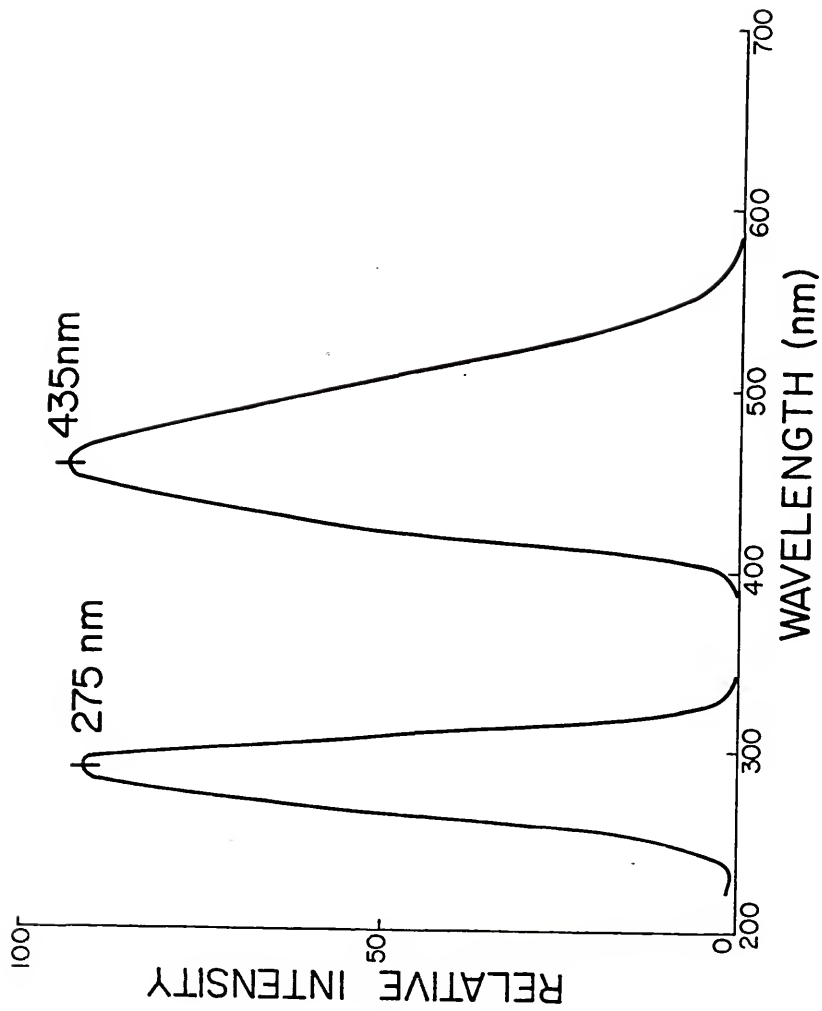
Additional molecular stabilization appears to occur when hydroxyl groups are present on the xanthine structure. This added stabilization may be attributed to the interactions (electrostatic and hydrogen bonding) of the hydroxyl groups on the molecules with the hydroxyl groups on the surface of the cellulose substrate material. Then, as would be expected, β-hydroxyethyltheophylline and dyphylline exhibit more intense RTP than caffeine. However, one would expect dyphylline (with 2 hydroxyl groups) to exhibit more intense RTP than β-hydroxyethyltheophylline. In this case, the reverse is true. Thus, in addition to the presence of methyl and hydroxyl groups on the xanthine structure, there are other unknown factors affecting the observed trends in phosphorescence intensity.

With 8-chlorotheophylline and the uric acid series, no RTP is observed. The presence of electron-withdrawing species (Cl and O) at position X_4 may contribute to resonance destabilization of the molecules which in turn could destabilize the triplet states of the molecules so that no RTP is observed. No other xanthine derivatives with electron-withdrawing groups (at positions other than X_4) were available to further test the destabilization theory.

While the observed trends of phosphorescence intensity within the xanthine groups are definitive, there is only a 20-fold difference in phosphorescence intensity in going from the weakest phosphor (xanthine) to the strongest phosphor (β-hydroxyethyltheophylline). Overall, this group of compounds represents an interesting series for persons wishing to study phosphorescence mechanisms. In contrast to observed differences in phosphorescence intensity, the xanthines give essentially the same RTP spectrum. A generalized RTP spectrum of the xanthine group (giving nominal wavelengths for the series) is represented in Figure 24.

Indoles. The differences in phosphorescence intensity within the indole group are more pronounced than within the xanthine group. The phosphorescence characteristics of the indole group are apparently determined by the relative solubility of the compounds in the solvent system. The first group (5-hydroxytryptophan, serotonin, and tryptophan)

Figure 24. Generalized RTP spectrum (nominal wavelengths shown) of the compounds in the xanthine series.


RELATIVE INTENSITY
100
50
0
200
300
400
500
600
700
WAVELENGTH (nm)
275 nm
435nm

is more soluble in water than the second group (5-hydroxy-indoleacetic acid, indole, indoleacetic acid, indole carboxylic acid, indomethacin and melatonin). The enhanced solvation (by water) of the molecules in the first group could allow the molecules to interact (via electrostatic interactions or hydrogen bonding) more effectively with the hydroxyl groups on the surface of the cellulose substrate material. These solvation effects could enhance the stability of the molecules adsorbed on the solid substrate whereby the triplet states become stabilized and large phosphoresence intensities are observed. On the other hand, the compounds in the second group are poorly solvated by water and the molecules can not participate in strong interactions with the cellulose substrate materials. The molecules of the second group exhibit RTP but do so at much lower levels (∿25 times lower) than the first group.

While the two groups drastically vary in exhibiting RTP, there is little variation in phosphorescence intensity within each group. Despite the structural similarity between the two groups, there are differences in the RTP spectra. Figures 25 and 26 are RTP spectra obtained from compounds in the first and second groups, respectively. Nominal wavelengths are shown on each spectrum because there is a lack of spectral resolution within each group.

Benzoic acid derivatives. For this diverse group of compounds, several generalizations can be made. Using benzoic acid as the starting material, the addition of

Figure 25. Generalized RTP spectrum (nominal wavelengths shown) of the compounds in the indole series (group 1).

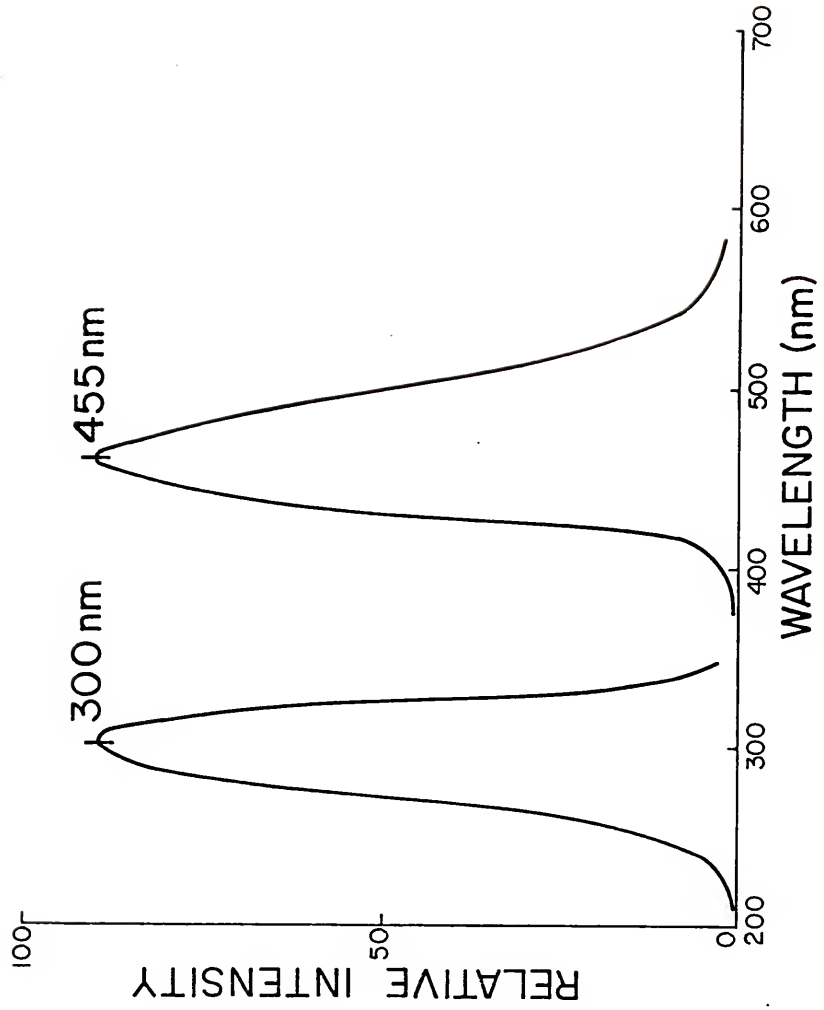
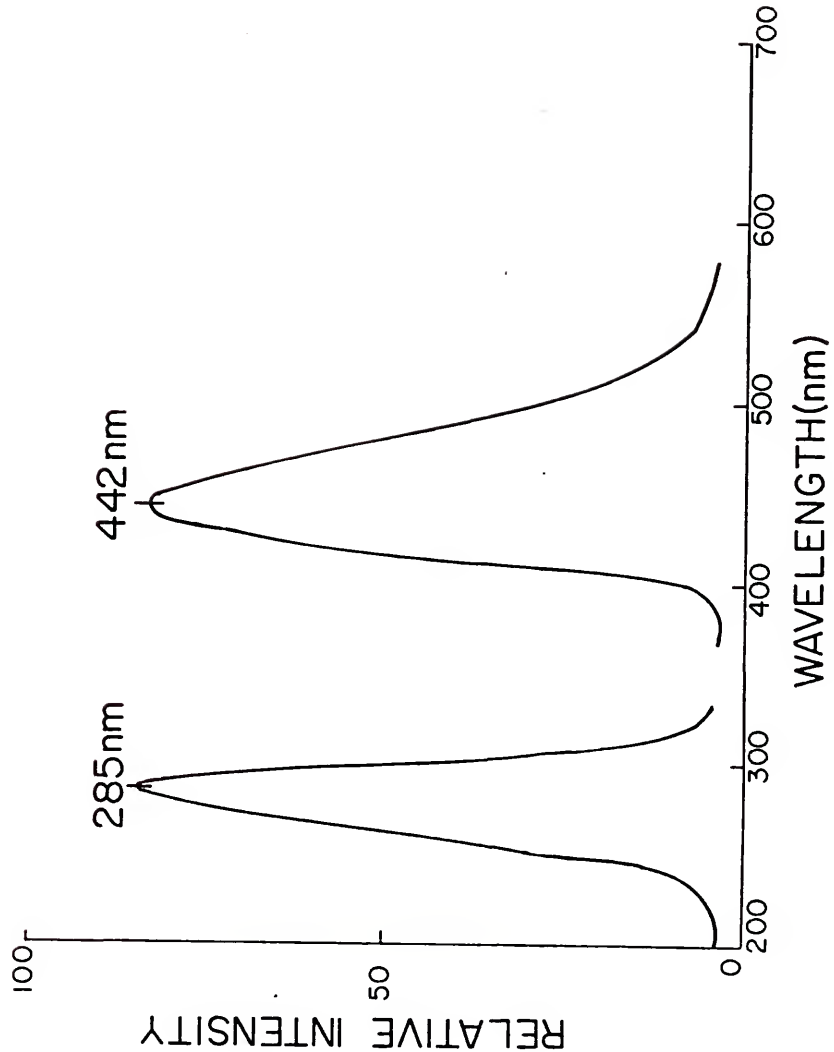

300nm
455nm
RELATIVE INTENSITY
100
50
0
200
300
400
500
600
700
WAVELENGTH (nm)

Figure 26. Generalized RTP spectrum (nominal wavelengths shown) of the compounds in the indole series (group 2).


RELATIVE INTENSITY
100
50
0
285nm
442nm
200
300
400
500
600
700
WAVELENGTH(nm)

certain groups to the benzene ring ortho and/or para to the carboxyl group greatly enhances the phosphorescence of the resultant compound. Group substitution meta to the carboxyl group on the benzene ring yields a species that exhibits RTP but the phosphorescence intensity is much lower than that of a compound with ortho/para substitution. Groups that are effective in providing resonance stabilization of the benzene ring can accommodate favorable interactions (electrostatic or hydrogen bonding) of the molecule with the cellulose substrate material. Appropriate groups include acetate, aldehyde, amide, hydroxyl and methoxy groups and amino and carboxy functions. The groups provide resonance stabilization of the benzene ring as well as contain atoms which can participate in hydrogen bonding or electrostatic interactions.

The most intense RTP is observed when an amino group is para to carboxy functions or when a hydroxyl group is ortho to an amino function or to a carboxy function. For the compounds studied in the group, trends in phosphorescence intensities can be expressed by the following relationships: p-aminobenzoic acid $\cong$ salicylamide > salicylic acid $\cong$ sulfosalicylic acid > acetylsalicylic acid $\cong$ procaine > p-aminosalicylic acid >> sulfacetamide $\cong$ vanillin > p-hydroxybenzoic acid > methylparaben $\cong$ p-aminohippuric acid > p-acetamidobenzoic acid $\cong$ propylparaben > probenecid. Lidocaine and benzoic acid do not exhibit RTP under the conditions used in these studies.

The difference in phosphorescence intensity between probenecid (weakest phosphor) and p-aminobenzoic acid (strongest phosphor) is a factor of ∿100. The phosphorescence intensity of p-aminobenzoic acid is ∿4 times that of sulfacetamide. RTP spectra obtained for some of the compounds in the benzoic acid derivative group are illustrated in Figures 27-34. In a mixture of strong phosphors, e.g., p-aminobenzoic acid and salicylamide, it is not possible to spectrally resolve the two components even though the excitation and emission maxima of the compounds differed. In general, it is difficult to resolve any one component in a multicomponent mixture of compounds in the benzoic acid group.

Others. The RTP studies of the remaining compounds yielded variable results. For compounds of similar chemical structure, it was found that compounds forming colored solutions upon dissolution in an appropriate solvent do not exhibit RTP while their respective chemical analogs, forming colorless solutions upon dissolution, do exhibit RTP. Two specific sets of compounds exemplify this point. In the first set, chloroquine exhibits intense RTP while primaquine, forming an orange-yellow-colored solution upon dissolution, does not phosphoresce. Folinic acid exhibits RTP; folic acid and methotrexate form yellow-colored solutions upon dissolution and do not phosphoresce. Folic acid, however, has been found to exhibit RTP when adsorbed on sodium acetate (67). The fact that folic acid does not

exhibit RTP when adsorbed on a cellulose-based support material further complicates the understanding of the phenomenon of RTP. Does the presence of color affect the observation of RTP? This is a complex question that can not be answered by the studies reported here. However, colored materials generally absorb light in the visible region of the light spectrum and this could be a reason for the lack of RTP. One other example of the presence of color and a lack of RTP involved the study of herbicides in Appendix 2. Most of the herbicides yielded yellow-colored solutions upon dissolution and did not exhibit RTP.

Several miscellaneous compounds were studied by RTP but no generalizations can be made regarding chemical structure and resulting phosphorescence intensities. As a matter of documentation, selected RTP spectra of various compounds are shown in Figures 35-42.

Two groups of compounds studied here gave results unlike other compounds with similar chemical structures. The catechol group (Figure 21) and the phenothiazine group (Figure 22) did not exhibit RTP. A few compounds in the catechol group exhibited RTP, but the intensity levels obtained from those compounds were not analytically useful. At this time, there is no available documentation to explain the results obtained for the catechol and phenothiazine groups. However, the compounds in question have been found to phosphoresce at low temperatures.

Figure 27. Room temperature phosphorescence spectrum of p-aminobenzoic acid.

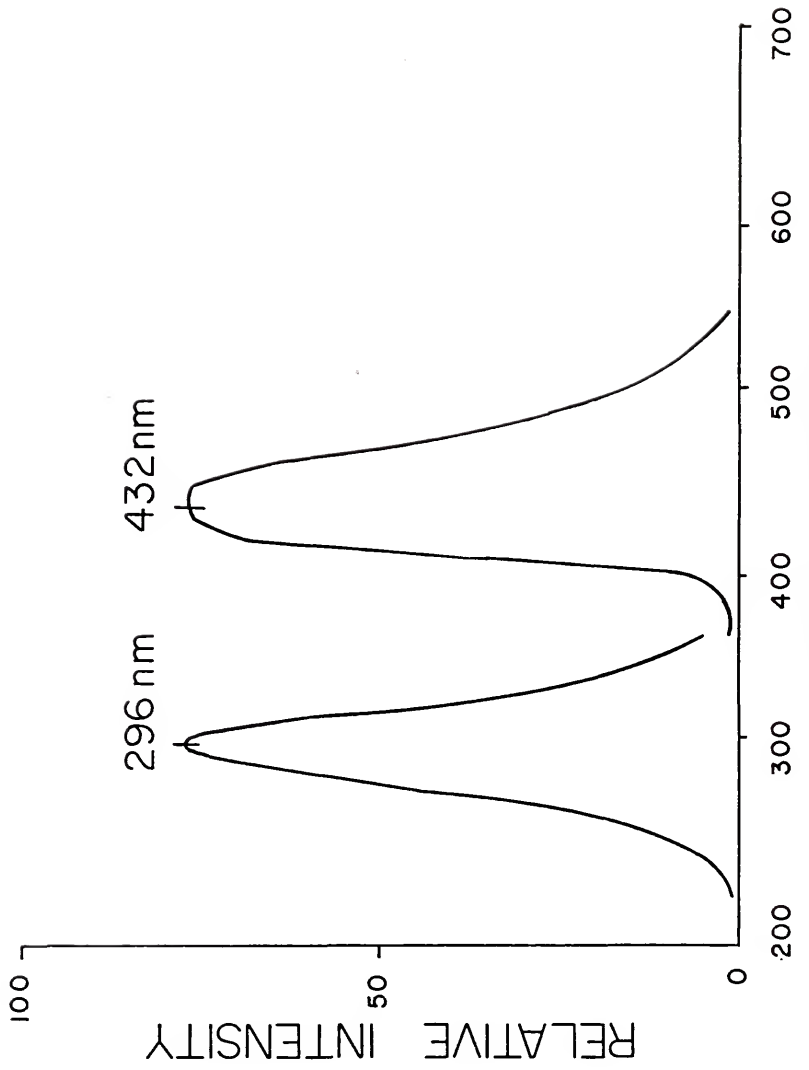

RELATIVE INTENSITY
100
50
0
200
300
400
500
600
700
WAVELENGTH(nm)
296 nm
432nm

Figure 28. Generalized RTP spectrum (nominal wavelengths shown) of acetylsalicylic acid, aminosalicylic acid, salicylamide, salicylic acid and sulfosalicylic acid.

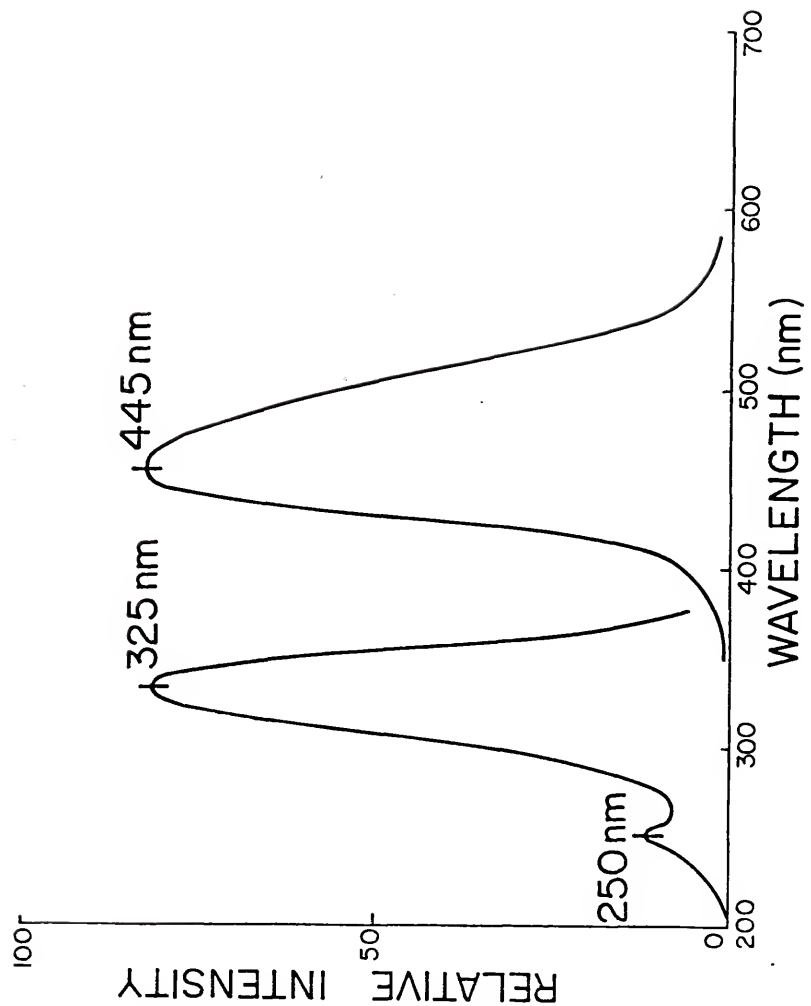
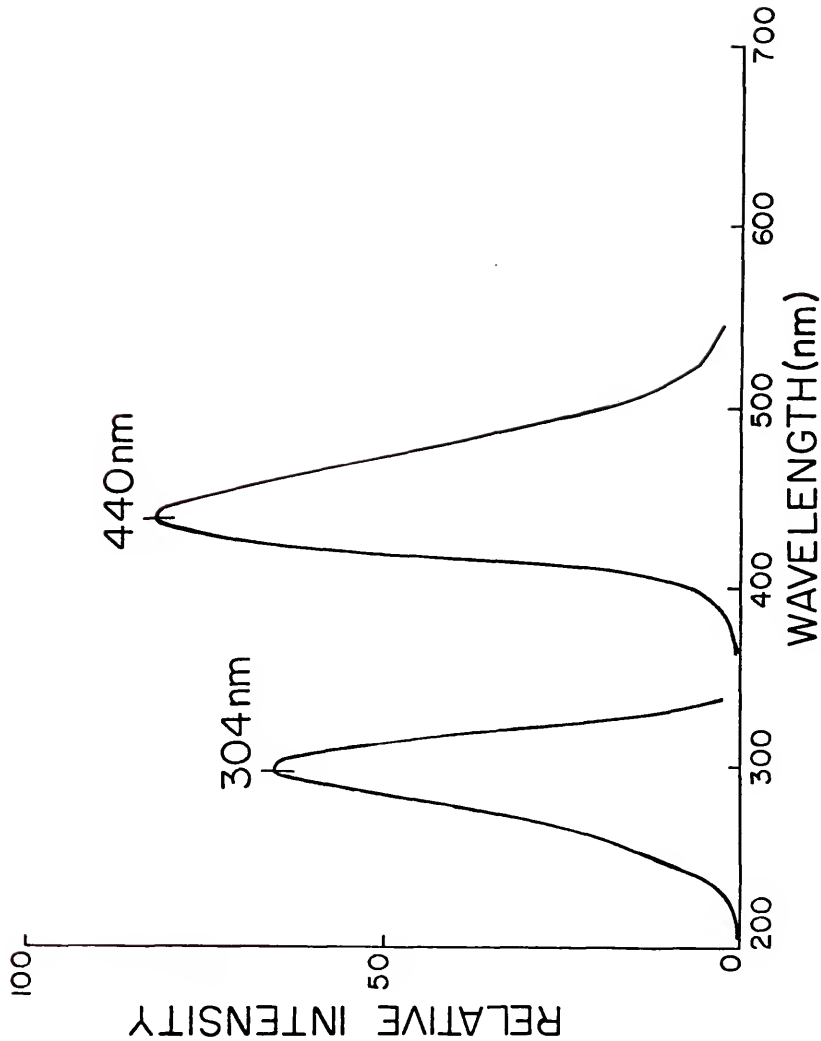

RELATIVE INTENSITY
100
50
0
200
300
400
500
600
700
WAVELENGTH (nm)
250 nm
325 nm
445 nm

Figure 29. Room temperature phosphorescence spectrum of procaine.


RELATIVE INTENSITY
100
50
0
200
300
400
500
600
700
WAVELENGTH(nm)
304nm
440nm

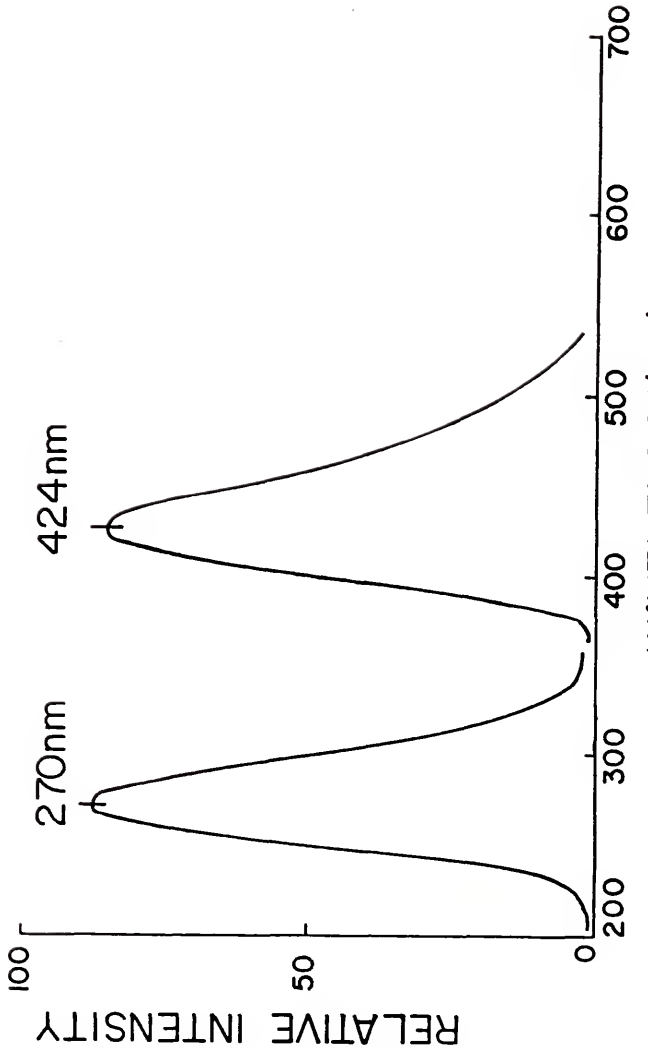


Figure 30. Room temperature phosphorescence spectrum of sulfacetamide.

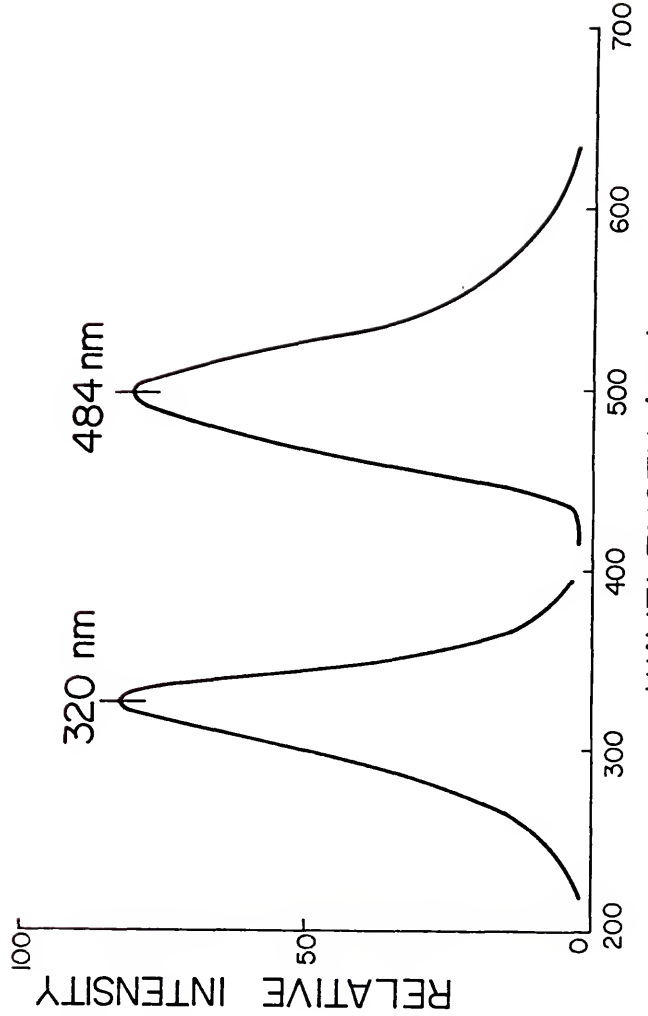


Figure 31. Room temperature phosphorescence spectrum of vanillin.

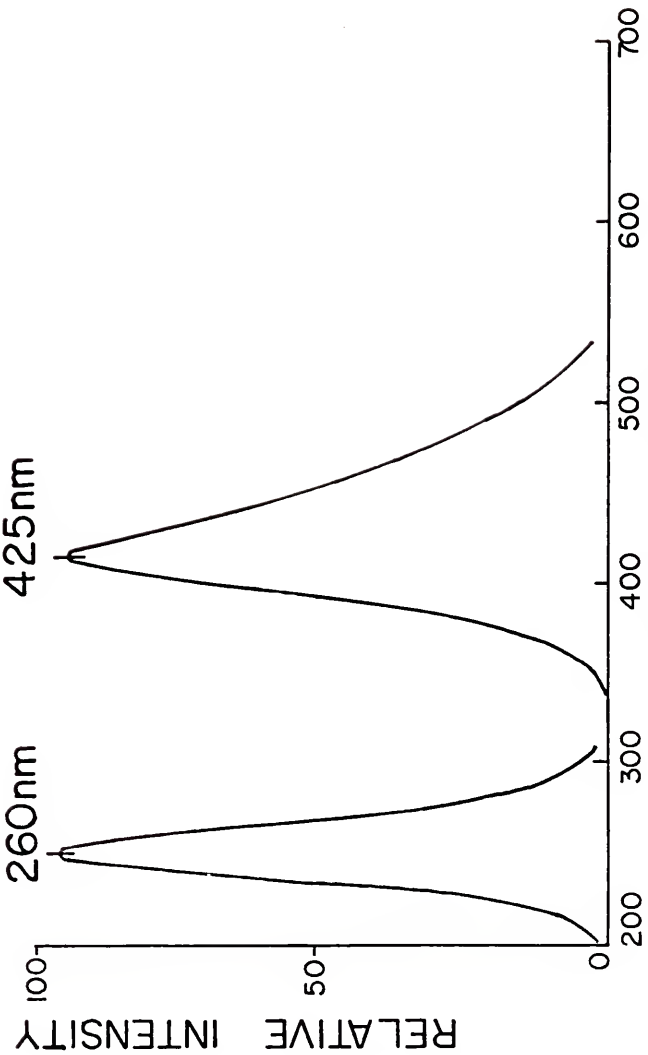


Figure 32. Generalized RTP spectrum (nominal wavelengths shown) of p-hydroxybenzoic acid, methylparaben and propylparaben.

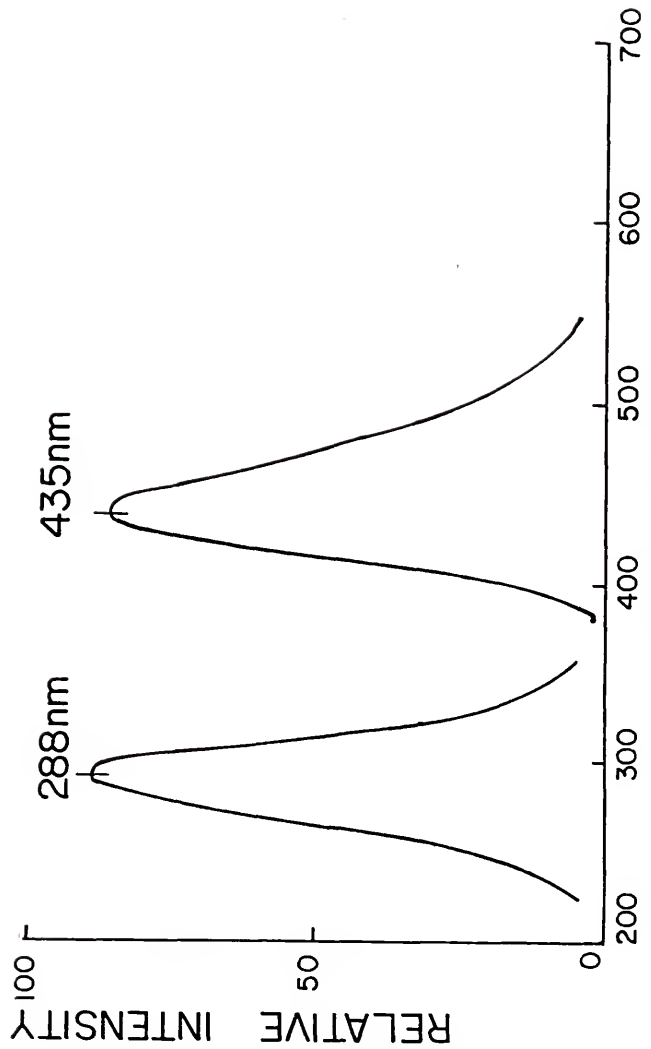


Figure 33. Generalized RTP spectrum (nominal wavelengths shown) of acetamidobenzoic acid and p-aminohippuric acid.

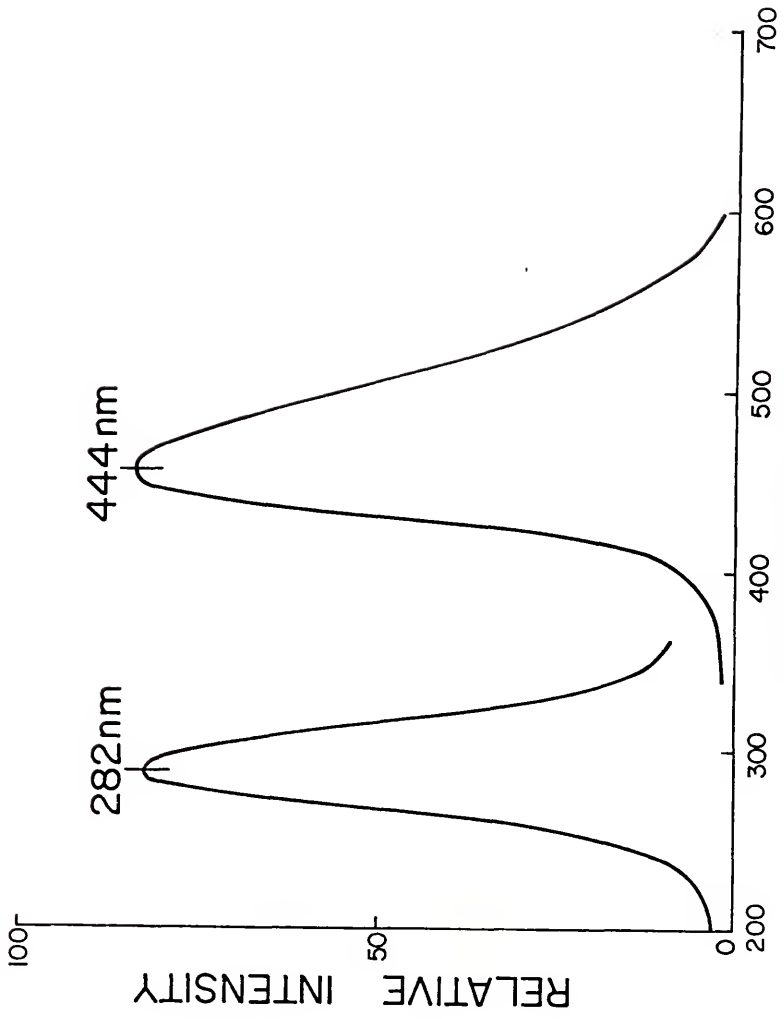


Figure 34. Room temperature phosphorescence spectrum of probenecid.

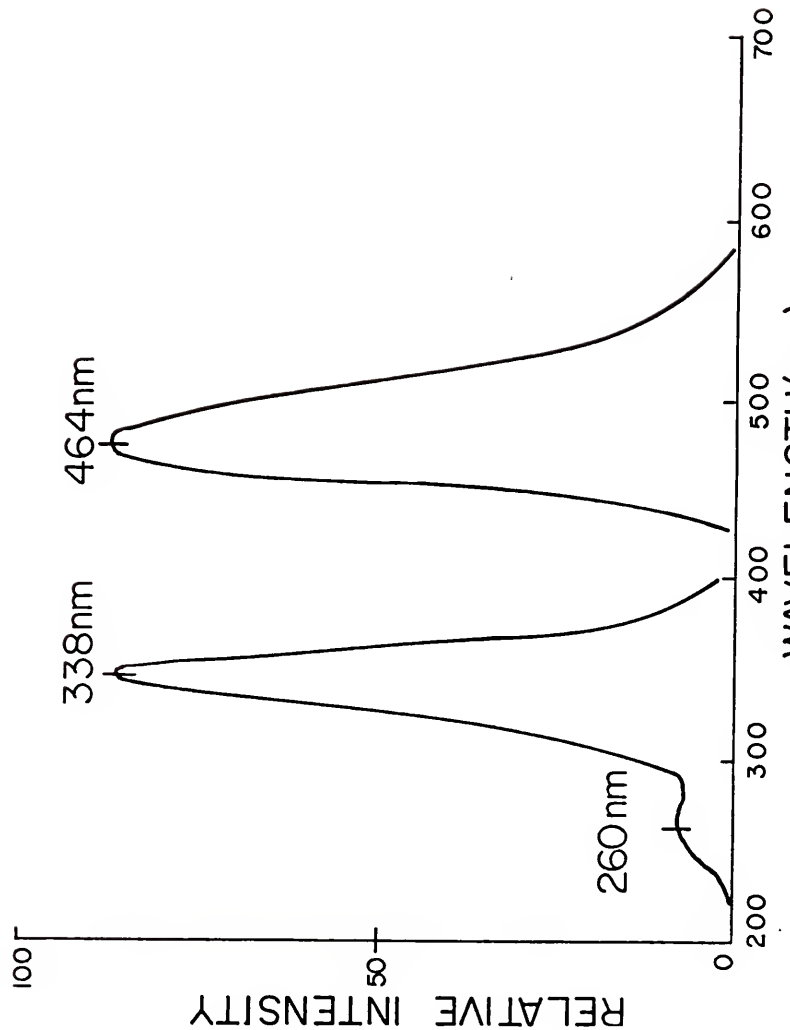
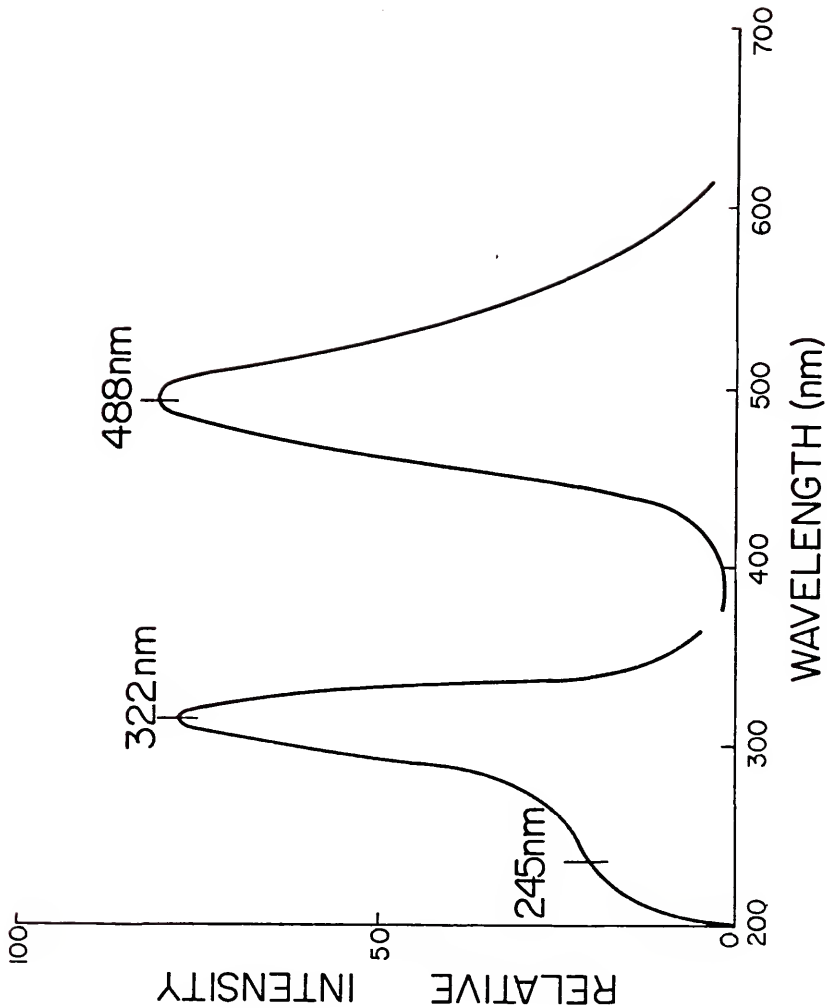


Figure 35. Room temperature phosphorescence spectrum of chloroquine.

Figure 36. Room temperature phosphorescence spectrum of dibucaine.


RELATIVE INTENSITY
100
50
0
200
300
400
500
600
700
WAVELENGTH (nm)
488nm
322nm
245nm

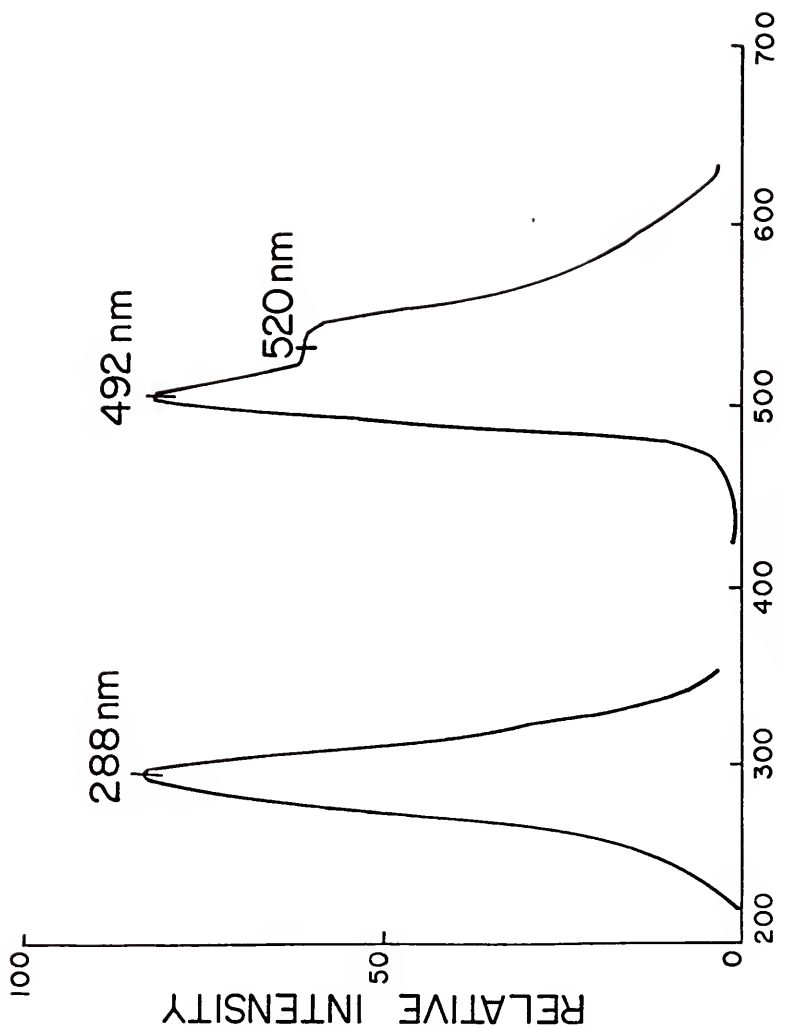


Figure 37. Room temperature phosphorescence spectrum of naphazoline.

Figure 38. Room temperature phosphorescence spectrum of propranolol or a mixture of propranolol and hydrochlorthiazide without the addition of sodium hydroxide.

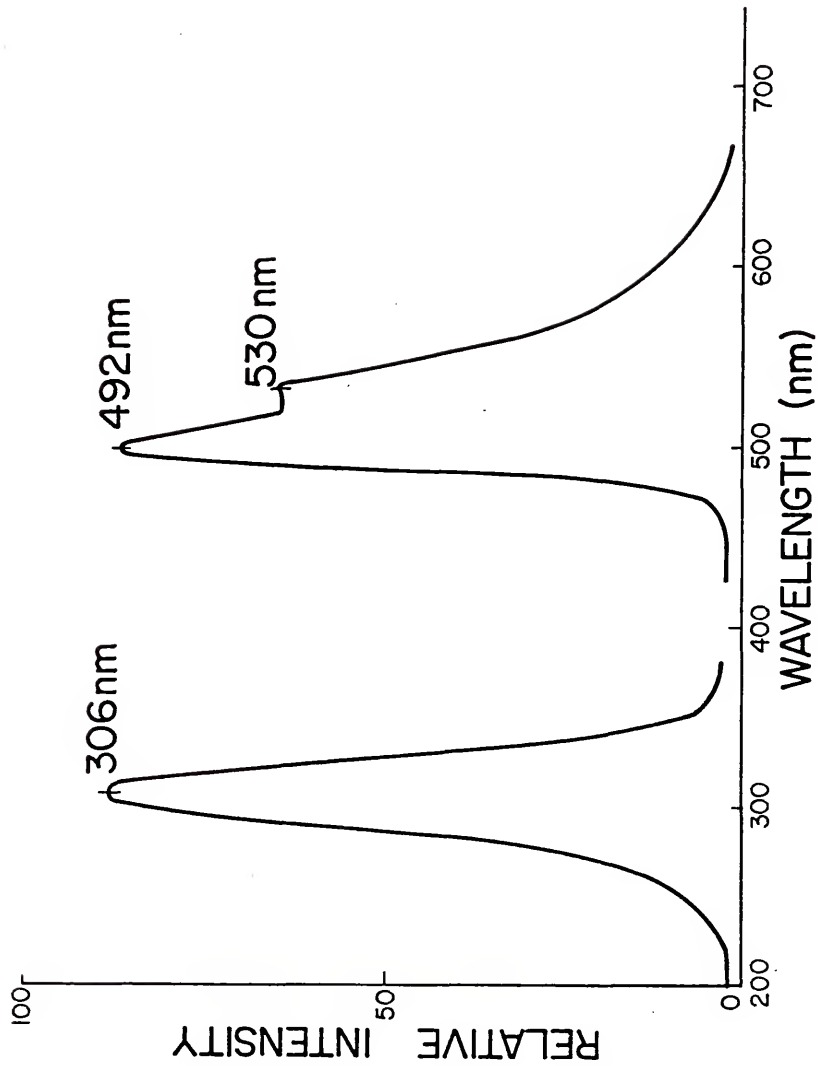
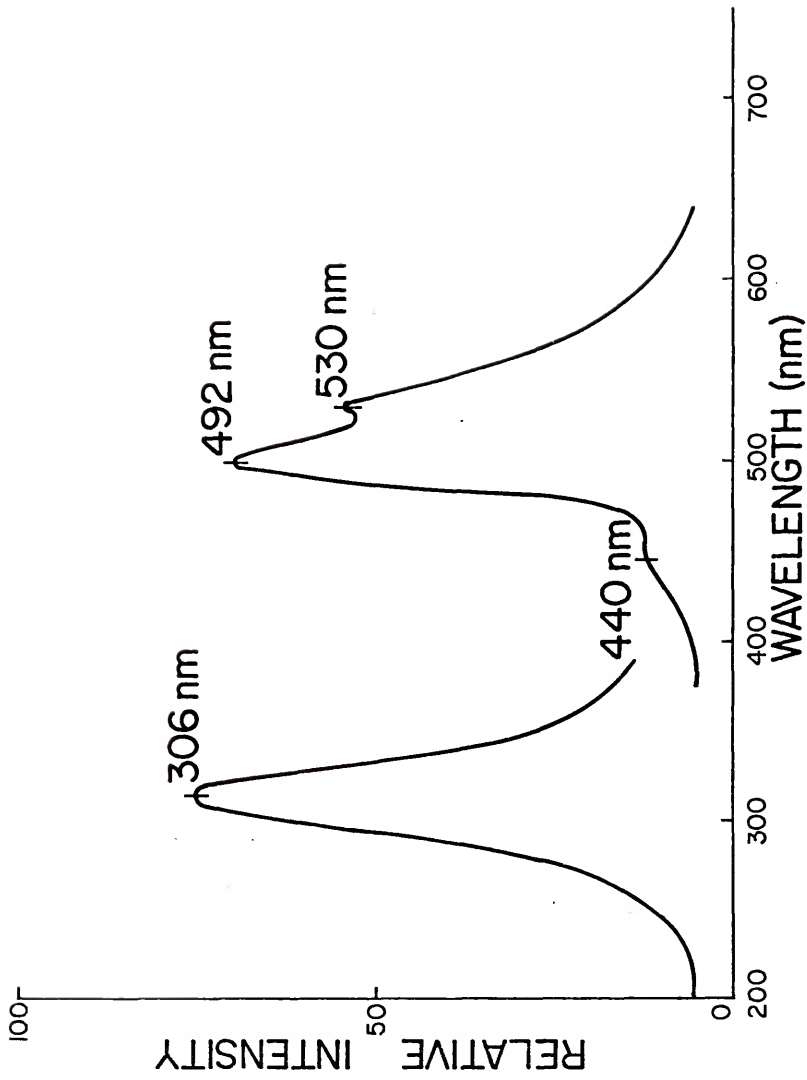

306nm
492nm
530nm
RELATIVE INTENSITY
100
50
0
200
300
400
500
600
700
WAVELENGTH (nm)

Figure 39. Room temperature phosphorescence spectrum of a mixture of propranolol and hydrochlorthiazide with the addition of sodium hydroxide.


RELATIVE INTENSITY
100
50
0
200
300
400
500
600
700
WAVELENGTH (nm)
306 nm
440 nm
492 nm
530 nm

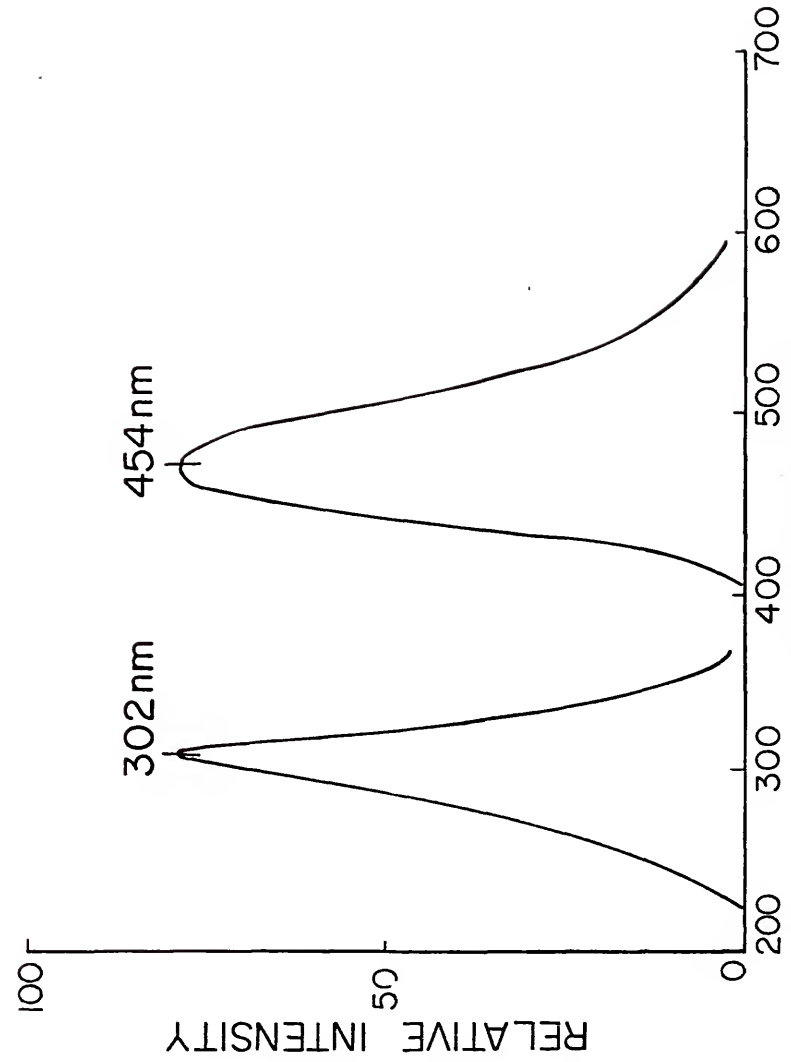


Figure 40. Room temperature phosphorescence spectrum of reserpine.

Figure 41. Room temperature phosphorescence spectrum of tetracycline.

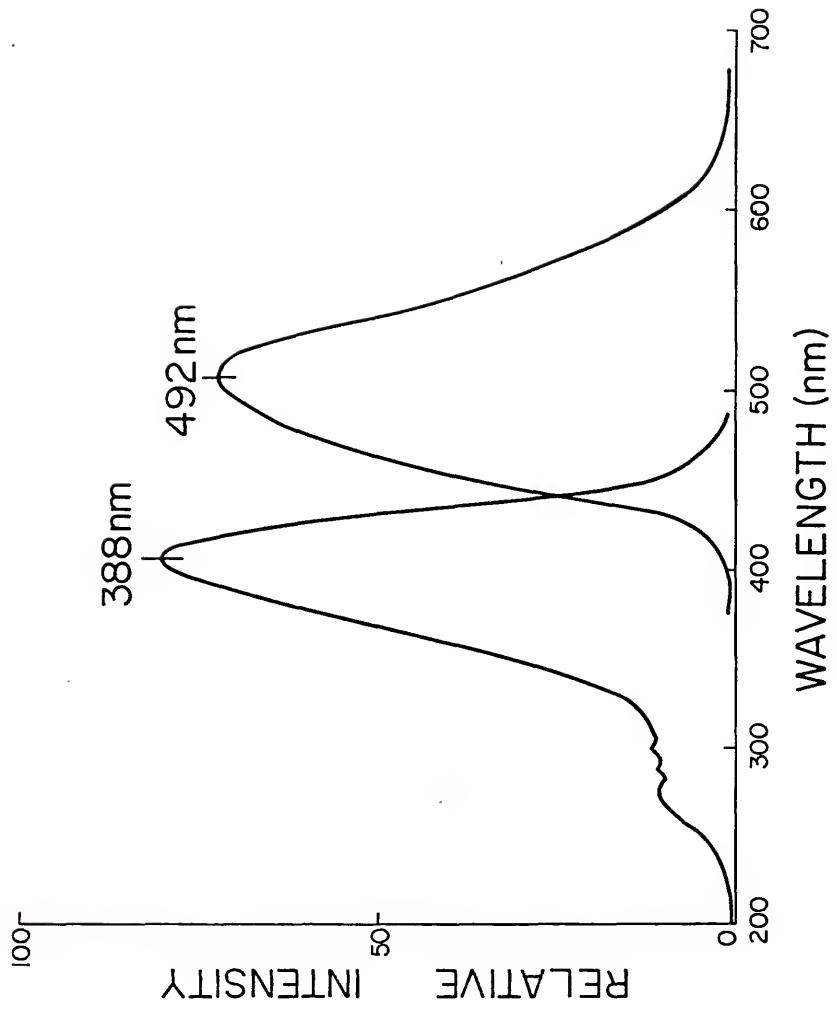
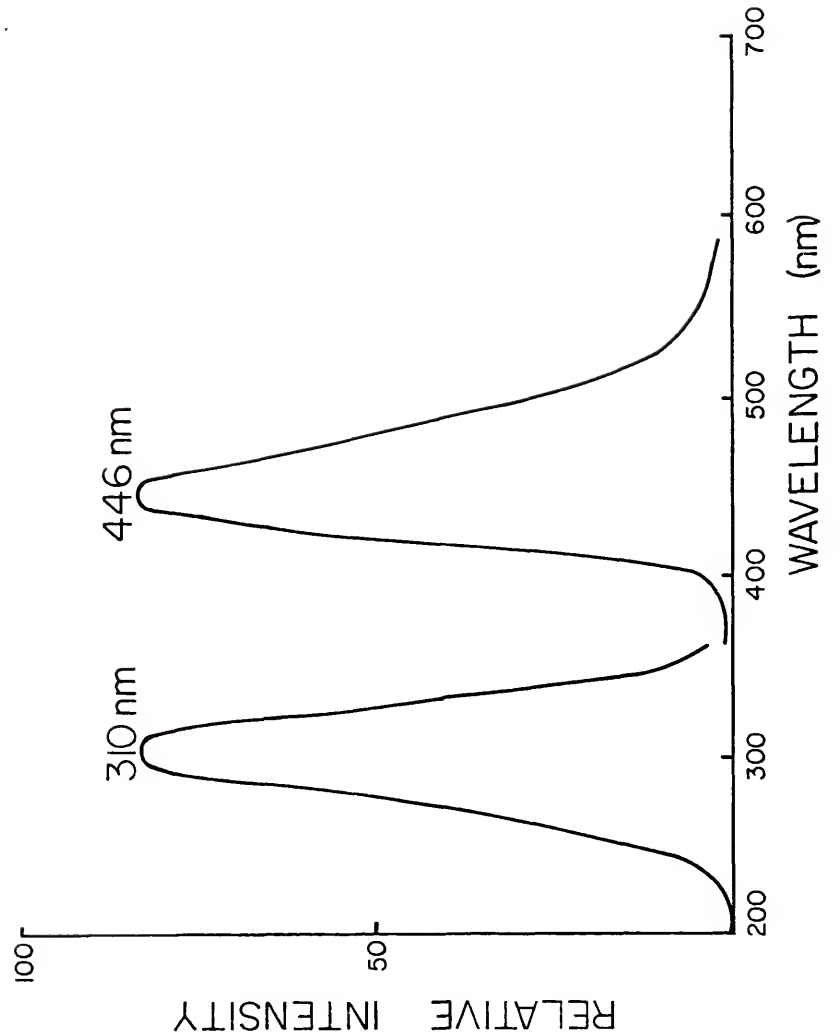

388nm
492nm
RELATIVE INTENSITY
100
50
0
200
300
400
500
600
700
WAVELENGTH (nm)

Figure 42. Room temperature phosphorescence spectrum of folinic acid.


RELATIVE INTENSITY
100
50
200
300
400
500
600
700
WAVELENGTH (nm)
310 nm
446 nm

Pharmaceutical Analysis

Xanthines. Having completed an in-depth study of a variety of compounds, it was next possible to evaluate RTP in its application to real sample analysis. In this first category, aminophylline, dyphylline, oxtriphylline and theophylline were analyzed in a variety of pharmaceutical formulations. For the analysis, capsules, compressed tablets, enteric-coated tablets, elixirs and syrups were assayed for active ingredients. The results for this study can be found in Tables 4-4 and 4-5. With the exception of Slo-Phyllin® and Accurbron™ , the results agreed quite-well with U.S.P. formulation analysis guidelines. U.S.P. guidelines dictate 90-110% recovery of the active ingredient in pharmaceutical formulations. Recovery studies performed with RTP yielded results that were within the specified limits established by U.S.P. guidelines.

Analgesics. For the analysis of acetylsalicylic acid (ASA) in several over-the-counter analgesic preparations, the results of the study can be found in Table 4-6. All of the experimental results, with the exception of those for BC® Powder, agree (within prescribed limits) with the manufacturers' specifications. BC® Powder contains ASA and salicylamide. In previous studies, it was noted that salicylamide exhibited more intense RTP than did ASA. In addition, ASA and salicylamide could not be spectrally resolved. In this study, the analysis of BC® Powder was performed using only ASA standards. Since no

Table 4-4. Results for the Determination of Several Xanthines in Pharmaceutical Formulations

Sample[A]	Concentration Found Experimental (µg/mL)[B]		Coefficient of Variation (%)
	Mean	Range	
Aminophyllin[a]	100	91-111	5.8
Asbron G® Inlay-Tabs®[d]	98	96-102	2.1
Bronkotabs®[d]	95	91-100	2.8
Choledyl®[c]	94	81-112	8.8
Lufyllin®-GG[b]	95	87-102	6.5
Quadrinal™[d]	94	91-99	2.8
Slo-Phyllin® 60 Gyrocaps®[d]	95	88-101	4.4
Theo-Dur®[d]	102	94-109	4.5
Slo-Phyllin®-80 Syrup[d]	114	109-120	3.0
Elixophyllin® Elixir[d]	98	91-103	3.6
Accurbron™[d]	125	119-133	3.1

(A) All test samples were 100 µg/mL active ingredient. Slo-Phyllin®-80 and Accurbron™ contained 107 µg/mL active ingredient. The last 3 samples are liquid preparations.

(B) Calculated from 16 measurements of each of 4 samples when compared to standard calibration curves.
Linear Dynamic Ranges: 5-300 µg/mL

[a]Aminophylline: $Y = 0.43 X + 0.37$

[b]Dyphylline: $Y = 1.0 X + 1.5$

[c]Oxtriphylline: $Y = 0.42 X + 0.55$

[d]Theophylline: $Y = 0.60 X + 0.56$

Correlation coefficients: $r = 0.999^{+}$

Table 4-5. Summary of Analysis of Several Xanthines in Solid Dosage Forms

Sample[A]	Labeled Amount (mg) of Active Ingredient/Dosage[B]	Experimental Amount (mg) of Active Ingredient/Dosage[C]
Aminophyllin	100	100
Asbron G® Inlay-Tabs®	150	147
Bronkotabs®	100	95
Choledyl®	100	94
Lufyllin®-GG	200	190
Quadrinal™	63	59
Slo-Phyllin® 60 Gyrocaps®	60	57
Theo-Dur®	200	204

(A) Quadrinal™ is corrected for theophylline weight equivalence in theophylline calcium salicylate. Active ingredients are cross-referenced in Table 4-1.

(B) Amount of active ingredient listed on container.

(C) Mean value calculated from 16 determinations of active ingredients in each weighed portion.

Table 4-6. Results for the Determination of Acetylsalicylic Acid (ASA) in Analgesic Preparations

Preparation (mg ASA/Dosage Form) (A)	Concentration Found Experimental (µg/mL) (B)		Coefficient of Variation (%)	Experimental Amount (mg) of ASA/Dosage Form (C)
	Mean	Range		
Anacin® (400)	103	97-109	3.2	412
Bayer® Aspirin (325)	101	96-106	3.4	328
BC® Powder (845)	138	134-142	2.4	1166
Bufferin® (324)	98	89-103	4.2	318
Empirin® (325)	94	87-101	3.9	305
Excedrin® (250)	101	92-106	4.4	252
Wyeth® Aspirin (300)	101	92-106	3.7	303

(A) Amount ASA listed on container. BC® powder has combined weight (mg) of ASA and salicylamide (650 and 195, respectively). All test samples were 100 µg/mL ASA except for BC® powder which was 130 µg/mL ASA and salicylamide.

(B) Calculated from 16 measurements of each of 4 samples when compared to standard calibration curve.

(C) Mean value calculated from 16 determinations of ASA in each of 4 weighed portions.
Linear Dynamic Range: 10-200 µg/mL
ASA: $Y = 16.5 X + 59$
Correlation coefficient: $r = 0.999^{+}$

correction factor was imposed to compensate for the differences in phosphorescence intensities between ASA and salicylamide, the experimental results indicated an overestimation of the assayed value for BC® Powder.

Others. To further evaluate the use of RTP in real sample analysis, additional pharmaceutical formulations and commercial preparations were assayed for a variety of active ingredients. The results of these studies can be found in Tables 4-7 and 4-8. The analysis of propranolol in Inderal® and Inderide® yielded excellent results. Hydrochlorthiazide, present in the Inderide® formulations, was not assayed because phosphorescence signals were too low to allow quantitation of hydrochlorthiazide as an active ingredient. Similar analyses of caffeine, chloroquine, naphazoline, p-aminobenzoic acid (PABA), reserpine and theobromine in formulations and preparations gave equally-good results.

Summary

In this chapter, an extensive list of biochemicals and drugs was established and the compounds were measured by RTP to illustrate the general applicability of RTP to chemical analysis. Of the 134 compounds studied by RTP, 54 compounds phosphoresced under the specified conditions. Of those 54 compounds, 9 compounds were labeled as not being analytically useful in RTP studies because of low signal levels.

Table 4-7. Results for the Determination of Propranolol in Pharmaceutical Formulations

(mg)	Formulation Active Ingredient/ Dosage Form[A]	Concentration Found Experimental (µg/mL)[B]		Coefficient of Variation (%)	Experimental Amount (mg) of Active Ingredient/ Dosage Form[C]
		Mean	Range		
	Inderal®				
	20	98	90-108	5.2	20
	40	97	88-108	7.2	39
	80	102	94-108	4.0	82
	1	103	94-111	4.4	1
	Inderide®				
	40/25	102	94-110	5.0	41
	80/25	101	94-110	4.5	81

(A) All test samples were 100 µg/mL active ingredient. Inderal® 1 is a 1 mL injectable ampul. Inderide® contains propranolol/hydrochlorthiazide.

(B) Calculated from 16 measurements of each of 4 samples when compared to standard calibration curve.

(C) Mean value calculated from 16 determinations of active ingredients in each of 4 weighed portions.
Linear Dynamic Range: 20-300 µg/mL
Propranolol: $Y = 0.725 X - 7.34$
Correlation coefficient: $r = 0.999^{+}$

Table 4-8. Results for the Determination of Several Active Ingredients in Pharmaceutical Formulations and Commercial Preparations

Formulation (Preparation) (mg Active Ingredient/ Dosage Form)(A)	Concentration Found Experimental (µg/mL)(B)		Coefficient of Variation (%)	Experimental Amount (mg) of Active Ingredient/ Dosage Form(C)
	Mean	Range		
Aralen® (300)[b]	102	94-111	4.7	306
Athemol® (74)[f]	103	100-106	1.9	77
NoDoz® (100)[a]	99	89-108	5.2	99
Pabanol™ (5% w/v)[d]	101	95-105	3.6	5.1%
Privine® (0.05% w/v)[c]	108	101-116	4.3	0.054%
Serpasil® (5)[e]	98	93-103	2.8	4.9
Vivarin® (200)[a]	100	91-108	5.0	200

(A) Athemol® is corrected for theobromine weight equivalence in theobromine magnesium oleate. Others are listed as amount of active ingredient listed on container. All test samples were 100 µg/mL active ingredient.

Table 4-8—continued.

(B) Calculated from 16 measurements of each of 4 samples.

(C) Mean value calculated from 16 determinations of active ingredients in each of 4 weighed portions.

Compound	Linear Dynamic Range	Line
[a]Caffeine	25-200 µg/mL	Y = 6.1 X - 5.6
[b]Chloroquine	25-200 µg/mL	Y = 9.5 X + 6.5
[c]Naphazoline	25-200 µg/mL	Y = 11.1 X + 1.9
[d]PABA	1-200 µg/mL	Y = 29 X - 10.1
[e]Reserpine	25-300 µg/mL	Y = 1.3 X + 2.7
[f]Theobromine	25-200 µg/mL	Y = 2.6 X + 7.0

Correlation coefficients: $r = 0.999^{+}$

Once the working list of compounds was established, RTP was successfully applied to the determination of active ingredients in pharmaceutical formulations and over-the-counter preparations. Because RTP has been well-established as a method of analysis with great selectivity, this selectivity advantage was used to illustrate the simplicity of RTP as a method of analysis of a variety of compounds in complex matrices.

CHAPTER 5
SUMMARY AND FUTURE CONSIDERATIONS

In retrospect of some diagnostic studies in RTP in its application to biochemical and drug analysis, several important points can be made. First, the evaluation of cellulose as a substrate material for RTP confirmed the presence of an inherent background phosphorescence of cellulose-based support materials and alluded to a probable source of the luminescence. The study also supported the choice of using cellulose-based support materials in RTP so long as the solid substrate was pre-treated to enhance its adsorption characteristics and to maximize signal-to-noise ratios.

Second, in view of the selectivity advantage of RTP, one could infer that the technique suffers from few interferences. However, studies made in the biochemical/drug survey indicated that RTP could not spectrally-resolve a series of compounds of similar chemical structure. Despite this lack of spectral resolution, it might be possible to use RTP in the time-resolved mode to characterize and effectively isolate molecules of similar chemical structure. Thus, in order to use the selectivity advantage of RTP to its fullest potential, it will be necessary to evaluate the use of time-resolved phosphorimetry (TRP) in

compensating for spectral-selectivity limitations inherent in RTP and in correcting for the background luminescence characteristic of cellulose-based substrate materials. Although the advantages of TRP have been know for over a decade, the technique has only recently become available on a commercial basis (Perkin-Elmer LS-5, June 1981).

The use of RTP in real-sample analysis was successful in analyzing for a variety of drugs in pharmaceutical formulations and over-the-counter preparations. Other situations for which RTP could be applied to real-sample analysis include the quantitation of p-aminobenzoic acid in urine for the assessment of pancreatic function and the use of RTP in solid-surface luminescence analysis of drugs on chromatogram-type substrates. These test methodologies are currently being considered and will hopefully be successfully carried out using the LS-5 spectrometer.

In the future, more research will be needed to evaluate additional compounds for RTP studies. Moreover, researchers doing work with RTP should standardize analytical procedures. The use of standardized procedures in RTP will hopefully compensate for variations between laboratories (and personnel) and will thus allow RTP to be more generally applicable as a method of chemical analysis.

APPENDIX 1
GLOSSARY FOR CHAPTER 3

Basis weight. The mass per unit area (g/m^2) of paper. Generally, however, in the paper industry it is the weight (lbs.) of a ream (usually 500 sheets) of paper cut to its basic size (filter paper - 20 x 20 in).

Cellulose. A linear polysaccharide, β-1,4 glucan, of high molecular weight.

Fibrillation. A term associated with refining of pulp that results in the loosening of threadlike "elements" from the fiber wall to provide greater surface area for forming fiber-to-fiber bonds.

Filter paper. A porous, unsized paper used in filtering solid particles from fluids. It is made from cotton fiber and/or wood pulp. Uniformity of formation is an important property. Analytical filter papers (qualitative and quantitative) have variable filtering rates; thus, pore size is carefully controlled to determine the speed (slow, medium or fast) of filtration. Quantitative filter paper must have a very low "ash content" (percentage of inorganic residue-based on the weight of the specimen-obtained by igniting a specimen of cellulose material in such a way that the combustible and volatile compounds are removed), which is achieved by washing with hydrochloric and hydrofluoric acids.

Gap. Voids present in cellulose sheets arising from "random" fiber orientation during sheet formation.

Handsheet. A sheet of paper made from a suspension of fibers in water. The sheetmaking operation is not a continuous process and each sheet is formed separately by draining a pulp suspension on a stationary mold. It is used for testing the physical and chemical properties of a pulp. It is formed in accordance with standard procedures to eliminate variables that affect results.

Hemicelluloses. Any of a number of cell-wall polysaccharides. They are usually not extractable by water or by most organic solvents. However, they are gradually extracted by dilute (∿10%) aqueous-alkali solutions. Other extractants may also be available.

Lignin. The non-carbohydrate portion of cell walls. It is an amorphous material of high molecular weight, predominantly aromatic in nature, built up of phenylpropane units. Lignin is not a compound, but rather it is a system and varies in composition with method of isolation and with species, age, growing conditions, etc. of the plant or tree. Lignin is almost completely removed during chemical pulping processing.

Lint. The ginned cotton textile fiber.

Linters. Short fibers adhering to the cottonseed after the ginning operation. The linters are removed in varying manners to give different "cuts" of fiber.

Porosity. The property of a substance containing connected voids. It is dependent upon the number, size, shape and orientation of voids. It is evaluated by air permeability, which is a property of a sheet which allows the passage of air when a pressure differential exists across its boundaries. It is evaluated by obtaining the flow rate of air through a specimen of given dimensions under standard conditions of pressure, pressure difference, temperature and relative humidity.

Pulp. Fiber material of one kind or another classified according to specimen type (wood, rag, cotton linters, etc.) and to manufacturing process (mechanical, semi-chemical, chemical, etc.; soda, acid sulfite, neutral sulfite, sulfate, kraft, etc.; unbleached, semi-bleached, or bleached).

Thickness. A dimension (usually expressed in thousandths of an inch) of a single sheet of paper when measured under specific conditions of area and pressure.

APPENDIX 2
ANCILLARY STUDIES

Herbicide Survey

Introduction

Herbicides, because of their widespread use to control plant growth, have been studied extensively in recent years (68). At the same time, the number of analytical methods developed for investigating these compounds has increased (69). To study the applicability of RTP to herbicides, a selection of these compounds was made and measured.

Procedure

In the analysis of the compounds listed in Table A2-1, stock solutions (100 µg/mL in 50/50 v/v ethanol/water) were made and then analyzed by the format of solvent systems described in Chapter 4.

Results and Discussion

Of the herbicides listed in Table A2-1 only bentazon, dicamba, cyanazine, naptalam, paraquat and picloram phosphoresced. However, the phosphorescence of these compounds was not analytically useful by the criterion listed in Table 4-3. Overall, RTP is sensitivity-limited in the analysis of herbicides by the poor solubility characteristics of these compounds in aqueous-based solvent systems and by the fact that ultratrace levels of these compounds

Table A2-1. List of Herbicides used in RTP Studies

Compound	Compound
Alachlor	Fluometuron
Atrazine	Linuron
Benefin	MCPA
Bentazon	Metribuzin
Blazer	Naptalam
Bromacil	Paraquat
Bromoxynil	Pendimethalin
Chloramben	Picloram
Cyanazine	Profluralin
2,4-D	Pronamide
DCPA	Propachlor
Dicamba	Propanil
Diclofop-methyl	Simazine
Dinoseb	2,4,5-T
Diuron	Trifluralin

Source: Environmental Protection Agency Repository, Research Triangle Park, NC.

exist in the environment. Organic-based solvent systems (e.g., hexane, isooctane and toluene) were not evaluated for the analysis of herbicides by RTP due to the untoward effects (primarily dissolution of the cellulose matrix) of these solvents on filter paper.

Therapeutic Drug Monitoring

Introduction

For several groups of drugs demonstrating significant correlation between blood serum/plasma levels and therapeutic effectiveness, therapeutic drug monitoring has gained acceptance as a reliable and practical approach to manage a patient's drug therapy. Sensitive and specific drug assays are needed to monitor drug levels in order to maintain a patient's therapeutic regimen. In past years, immunoassays and chromatographic methods of analysis have been favorably applied to the determination of therapeutic agents in biological fluids. In this study, RTP was used to analyze theophylline in quality control sera and patient samples.

Reagents and Materials

Quality control sera (Profile I and II, General Diagnostics, Division of Warner-Lambert), patient samples and Syva EMIT® procedures were complimentary of the Clinical Chemistry Laboratory at Shands Teaching Hospital. Standard sera (10, 20, 40, 80 and 100 µg/mL theophylline) were prepared using Sera Chem® (Fisher Scientific) as a base. All other analytical agents were previously described.

Procedure

Both standard and quality control sera and patient samples were treated in the following manner:

(1) Add 0.5 mL serum to 0.5 mL 0.5 M pH 7.4 phosphate buffer in an extraction tube.

(2) Extract mixture for 5 min with 5 mL 95/5 v/v chloroform/isopropanol.

(3) Collect ∿5 mL organic phase through Whatman 1 filter paper. Reduce to ∿2 mL by bubbling nitrogen gas into extraction tube.

(4) Add 0.5 mL 0.1 M NaOH to reduced volume. Extract for 5 min and then centrifuge at 1000 g for 2 min.

(5) Collect 0.4 mL aqueous phase, add 0.1 mL 10 M KI, 0.05 mL 4 M NH_4Cl and 0.05 mL 1 M HCl.

(6) Make RTP measurement.

Quantitation of the quality control sera and patient samples was achieved by comparing sample phosphorescence intensities to those of standards. All samples were then rechecked for accuracy with the Syva EMIT® procedure. However, no correlation studies were performed due to the expense of the reagent kits for the EMIT® procedure.

Results and Discussion

The therapeutic range of the theophylline for treatment of apnea and asthma is generally accepted as 10-20 µg/mL. In this study, the linear range for theophylline determinations was 20-100 µg/mL. Theophylline concentrations below 20 µg/mL were determinable but were done so with poor accuracy. Unfortunately, none of the samples

assayed for theophylline was greater in concentration than 20 μg/mL. No calculations were performed for this part of the study because any calculations made outside the linear range for theophylline would not be analytically useful.

The extraction procedure was slightly altered so that a preconcentration step could be evaluated. The use of Sep-pak^{TM} (C_{18}, Waters Associates) cartridges for sample preparation was evaluated for sample preconcentration. The extraction of theophylline from serum samples using the Sep-pak^{TM} cartridges was non-reproducible in that a variety of separation schemes using the cartridges did not effectively isolate theophylline from caffeine and theobromine. In addition, since larger sample volumes were required for sample preconcentration, this procedure suffered an inherent disadvantage.

For these studies, RTP was basically less sensitive than several other analytical techniques. However, RTP is a highly selective technique and therefore can be used successfully in areas where high sensitivity is not a requirement.

APPENDIX 3
COMPANY LIST

Company	City, State
American Instrument Co.	Urbana, IL
Ayerst Laboratories	New York, NY
Barnsted Sybron Corp.	Boston, MA
BC Remedy Co.	Memphis, TN
Berlex Laboratories, Inc.	Cedar Knolls, NJ
Breon Laboratories	New York, NY
Bristol-Myers Co.	New York, NY
Buckeye Cellulose Corp.	Memphis, TN
Burroughs Wellcome Co.	Research Triangle Park, NC
Canrad-Hanovia, Inc.	Newark, NJ
Ciba-Geigy Corp.	Summit, NJ
Dooner Laboratories, Inc.	Ft. Washington, PA
Dorsey Laboratories	Lincoln, NE
Dow Chemical USA	Indianapolis, IN
Eaton-Dikeman	Mt. Holly Springs, PA
Eimac Division of Varian	San Carlos, CA
P.B. Elder Co.	Bryan, OH
Fisher Scientific Co.	Pittsburgh, PA
Glaxo, Inc.	Ft. Lauderdale, FL
Glenbrook Laboratories	New York, NY
Hamamatsu Corp.	Middlesex, NJ
ITT-Rayonier	Stamford, CT
Key Pharmaceuticals, Inc.	Miami, FL
Knoll Pharmaceutical Co.	Whippany, NJ
Mallinckrodt, Inc.	Paris, KY
Perkin-Elmer Corp.	Oak Brook, IL

Company	City, State
Pfizer, Inc.	New York, NY
Riker Laboratories	Northridge, CA
Sandoz Pharmaceuticals	East Hanover, NJ
Schering Corp.	Kenilworth, NJ
Schleicher & Schuell, Inc.	Keene, NH
Schoeffel Instrument Div.	Westwood, NJ
Scientific Manufacturing Industries, Inc.	Emeryville, CA
G.D. Searle & Co.	Chicago, IL
Sigma Chemical Co.	St. Louis, MO
Smith Kline & French Laboratories	Philadelphia, PA
Sorenson	Norwalk, CT
Southern Cellulose Products	Chattanooga, TN
E.R. Squibb & Sons, Inc.	Princeton, NJ
Syva Co.	Palo Alto, CA
U.S. Industrial Chemical Co.	New York, NY
United States Pharmacopeia	Rockville, MD
Wallace Laboratories	Cranbury, NJ
Warner-Lambert Co.	Morris Plains, NJ
Waters Associates, Inc.	Milford, MA
Whatman Laboratory Products, Inc.	Clifton, NJ
Whitehall Laboratories Inc.	New York, NY
J.B. Williams Co., Inc.	Cranford, NJ
Winthrop Laboratories	New York, NY
Wyeth Laboratories	Philadelphia, PA

APPENDIX 4
DATA FOR LINEAR REGRESSION ANALYSIS

As an example, data are given here for linear regression analysis to complement Table 4-4. The ordinate values (Y) represent mean relative phosphorescence signals (mean of 16 measurements of each standard solution) and the abcissa values (X) represent concentrations (μg/mL) of standard solutions. The lines in Table 4-4 were obtained using a HP 34C calculator (Hewlett-Packard, Corvallis, OR). The correlation coefficients for the lines were: $r = 0.999^{+}$.

Aminophylline

X	Y
200	85.75
100	42.75
50	22.5
25	10.35
10	4.8

Dyphylline

X	Y
100	101.25
50	52.5
25	26.75
10	12.53
5	4.83

Oxtriphylline

X	Y
200	84.75
100	43.
50	21.9
25	10.35
10	5.1

Theophylline

X	Y
200	121.5
100	59.5
50	33.25
25	14.7
10	6.23

REFERENCES

1. R.J. Hurtubise, Solid Surface Luminescence Analysis: Theory, Instrumentation, Applications, Marcel Dekker, Inc., New York, 1981.

2. R.T. Parker, R.S. Freedlander and R.B. Dunlap, Anal. Chim. Acta, 119, 189 (1980).

3. R.T. Parker, R.S. Freedlander and R.B. Dunlap, Anal. Chim. Acta, 120, 1 (1980).

4. J.L. Ward, Ph.D. Thesis, University of Florida, Gainesville, FL, 1980.

5. E. Wiedemann, Ann. Physik, 34, 446 (1888).

6. G.N. Lewis and M. Kasha, J. Am. Chem. Soc., 66, 2100 (1944).

7. R.J. Kiers, R.D. Britt, Jr. and W.E. Wentworth, Anal. Chem., 29, 202 (1957).

8. D.M. Hercules, Anal. Chem., 38, 29A (1966).

9. C.M. O'Donnell and J.D. Winefordner, Clin. Chem., 21, 285 (1975).

10. C.M. O'Donnell and T.N. Solie, Anal. Chem., 48, 175R (1976).

11. J.D. Winefordner and P.A. St. John Anal. Chem., 35, 2211 (1963).

12. Y. Kanda and R. Shimada, Spectrochim. Acta, 17, 279 (1961).

13. H.C. Hollifield and J.D. Winefordner, Anal. Chem., 40, 1759 (1968).

14. R. Zweidinger and J.D. Winefordner, Anal. Chem., 42, 639 (1970).

15. J.L. Ward, R.P. Bateh and J.D. Winefordner, Appl. Spectrosc., 34, 15 (1980).

16. J.L. Ward, G.L. Walden, R.P. Bateh and J.D. Winefordner, Appl. Spectrosc., 34, 348 (1980).

17. C.M. Lewis, D. Lipkin and T.T. Magel, J. Am. Chem. Soc., 63, 3005 (1941).

18. G. Oster, N. Geactinov and T. Cassen, Acta Phys. Polon., 26, 489 (1964).

19. A. Szent-Györgi, Science, 126, 751 (1957).

20. M. Roth, J. Chromatogr., 30, 276 (1967).

21. E.M. Schulman and C. Walling, Science, 178, 53 (1972).

22. M. Kasha, J. Chem. Phys., 20, 71 (1952).

23. S.P. McGlynn, J. Daigre and F.J. Smith, J. Chem. Phys., 39, 675 (1963).

24. R.A. Paynter, S.L. Wellons and J.D. Winefordner, Anal. Chem., 46, 736 (1974).

25. S.L. Wellons, Ph.D. Thesis, University of Florida, Gainesville, FL, 1973.

26. E. Lue Yen-Bower, Ph.D. Thesis, University of Florida, Gainesville, FL, 1978.

27. J.L. Ward, R.P. Bateh and J.D. Winefordner, Analyst, 107, 335 (1982).

28. W. Baeyens (Rijks Universiteit, Ghent, Belgium), private communication.

29. H.E. Millson, The Phosphorescence of Textile Fibers and Other Substances, Calco Tech. Bull., 753, American Cyanamid Co., 1944.

30. E.N. Harvey and A.M. Chase, Rev. Sci. Instrum., 13, 365 (1942).

31. Chemical Abstracts, 48, 357h (1953).

32. E. Lue Yen-Bower, J.L. Ward, G. Walden and J.D. Winefordner, Talanta, 27, 38 (1980).

33. J.L. Ward, E. Lue Yen-Bower and J.D. Winefordner, Talanta, 28, 119 (1981).

34. The Dictionary of Paper, 3rd Ed., American Paper and Pulp Association, New York, 1965.

35. C.F. Cross and E.J. Bevan, A Text-book of Papermaking, 5th Ed., E. and F.N. Spon. Ltd., London, 1936.

36. E. Ott, H.M. Spurlin and M.W. Graffin, eds., Cellulose and Cellulose Derivatives, Pt. 2, Interscience Publishers, Inc., New York, 1954.

37. J.P. Casey, Pulp and Paper: Chemistry and Chemical Technology, Vol. 1, Interscience Publishers, Inc., New York, 1960.

38. W.B. Wheelwright, Practical Paper Technology, M.J. and W.B. Wheelwright, Publishers, Cambridge, 1952.

39. H.F. Rance, ed., Handbook of Paper Science, Vol. 1, Elsevier Scientific Publishing Co., New York, 1976.

40. J. d'A. Clark, Pulp Technology and Treatment for Paper, Miller Freeman Publications Inc., San Francisco, 1978.

41. B.L. Browning, Analysis of Paper, 2nd Ed., Marcel Dekker, Inc., New York, 1977.

42. R.W. Jenkins, Southern Pulp and Paper, May 1980.

43. Cotton Linters Pulp Manufacture, The Buckeye Cellulose Corporation Technical Memorandum, January 1979.

44. J.B.F. Lloyd and J.N. Miller, Talanta, 26, 180 (1979).

45. R.H. Atalla and S.C. Nagel, J.C.S. Chem. Comm., No. 19, 1049 (1972).

46. T.E. Timell (State University of New York), private communication.

47. H.R. Huwyler, G. Franz and H. Meier, Planta, 146, 635 (1979).

48. H.R. Huwyler, G. Franz and H. Meier, Plant Science Letters, 12, 55 (1978).

49. D.P. Delmer, U. Heiniger and C. Kulow, Plant Physiol., 59, 713 (1977).

50. L. Waterkeyn, Protoplasma, 106, 49 (1981).

51. D. Maltby, N.C. Carpita, D. Montezinos, C. Kulow and D.P. Kelmer, Plant Physiol., 63, 1158 (1979).

52. U. Heiniger and D.P. Delmer, Plant Physiol., 59, 719 (1977).

53. M.C. Meinert and D.P. Delmer, Plant Physiol., 59, 1088 (1977).

54. G. Faulkner, W.C. Kimmins and R.G. Brown, Can. J. Bot., 51, 1503 (1973).

55. M.M. Smith and M.E. McCully, Protoplasma, 95, 229 (1978).

56. W. Bell (Buckeye Cellulose), private communication.

57. M. Bateh (Pratt & Whitney, West Palm Beach, FL), private communication.

58. R.M.A. von Wandruszka and R.J. Hurtubise, Anal. Chem., 48, 1784 (1976).

59. F.A.F. Mohamed, Ph.D. Thesis, Rijks Universiteit, Ghent, Belgium, 1981.

60. R.P. Bateh and J.D. Winefordner, Anal. Lett., 15(B4), 373 (1982).

61. L.A. Gifford, J.N. Miller, D.L. Phillipps, D.T. Burns and J.W. Bridges, Anal. Chem., 47, 1699 (1975).

62. W.J. Spann, J.J. Mousa, J.J. Aaron and J.D. Winefordner, Anal. Biochem., 53(1), 154 (1973).

63. I. Rubin, Pharmacy Times, 48(4), 23 (1982).

64. United States Pharmacopeia XX/National Formulary XV, United States Pharmacopeial Convention, Rockville, MD, 1980.

65. American Drug Index, 25th Ed., J.P. Lippincott Co., Philadelphia, 1981.

66. The Merck Index, 9th Ed., Merck & Co., Inc. Rahway, 1976.

67. R.M.A. von Wandruszka and R.J. Hurtubise, Anal. Chim. Acta, 93, 331 (1977).

68. J.J. Aaron, E.M. Kaleel and J.D. Winefordner, J. Agric. Food Chem., 27(6), 1233 (1979).

69. H.J. Sanders, Chem. Eng. News, 59(31), 20 (1981).

BIOGRAPHICAL SKETCH

Ricky Phillip Bateh, was born April 26, 1956, in Jacksonville, Florida. After attending public schools in Jacksonville, he graduated from Wolfson Senior High School. He spent the next four years at the University of Florida, graduating with a Bachelor of Science degree in chemistry in June, 1978. Since September, 1978, Ricky has attended the University of Florida, working towards the Doctor of Philosophy degree.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

James D. Winefordner, Chairman
Professor of Chemistry

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

John G. Dorsey
Assistant Professor of Chemistry

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Andrawus I. Khuri
Associate Professor of Statistics

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

M. Luis Muga
Professor of Chemistry

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Richard A. Yost
Assistant Professor of Chemistry

This dissertation was submitted to the Graduate Faculty of the Department of Chemistry in the College of Liberal Arts and Sciences and to the Graduate Council, and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

August, 1982

Francis G. Stehli
Dean for Graduate Studies and Research